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branchial cavities, which communicate with the pharynx by the gill-slits, and the median dorsal cloaca, which leads from the peribranchial cavities to the exterior.

The commonest method of formation of the peribranchial cavities is by two lateral ectodermal invaginations, e. g. Clavelina, Ciona. In these cases the peribranchial invaginations remain separate even in the free-swimming larva, and it is not until after fixation that the two lateral openings move dorsally and join, either directly or by means of a median depression between them, to form the single dorsal cloacal aperture.

A second method of origin is that described in Molgula, Anurella, and Styelopsis. Instead of the lateral peribranchial cavities arising first and the cloaca subsequently, a single dorsal ectodermal ingrowth gives rise to a median vesicle from which the peribranchial chambers arise as secondary diverticula.

In 1893 Pizon claimed to show that in the embryos of Botryllids the peribranchial cavities arose as lateral outgrowths from the pharyngeal rudiment. Although this is the normal method by which the peribranchial cavities develop in the buds of Ascidians, we have conclusive evidence that Pizon's extension of this method to the embryonic development was based upon a number of inaccurate interpretations. In point of fact the atrium develops by means of a single dorsal invagination of ectoderm in a manner closely conformable to that already described for various Molgulids, but the method is complicated in Botrylloides by the previous development of a peculiar 'dorsal groove' to which reference has already been made. One of the chief sources of Pizon's errors was his confusion of this groove with an embryonic neural groove (1893, pp. 63, 64, 69, 70). He even gives a characteristic figure of the atrial vesicle arising from the ectodermal groove as a bilobed invagination, but mistakes it for a developing 'sensory vesicle' (l. c., p. 65, Pl. ix, fig. 87; compare with our Pl. 1, fig. 8, *b*).

With these preliminary remarks we can review the stages in the development of the atrium seriatim.

At an early stage of development (Stage II), when the nervous system has completely separated from the ectoderm, and is undergoing its earliest differentiation into parts (the otolith cell being still dorso-lateral), the ectoderm overlying the nerve-cord becomes thickened and flattened, and shows the faint beginnings of its conversion into a shallow longitudinal groove (Pl. 2, fig. 10). From front to back as far as the base of the tail the neural tube lies immediately underneath it, and the dorsal plate itself is devoid of any proliferation or outgrowth from the region of the cerebral vesicle backwards (cf. Pl. 1, fig. 5).

In the next stage (Stage III) the dorsal plate has become definitely incurved as the 'dorsal groove' (Pl. 1, fig. 8), and immediately behind the cerebral vesicle and ganglion (Pl. 1, fig. 8, *a*) its groove suddenly deepens as the cavity of a well-marked open involution (Pl. 1, fig. 8, *b*). This is the combined atrio-cloacal vesicle. It is directed somewhat backwards in the sagittal plane, and develops a right and left lobe at its distal extremity, the rudiments of the right and left peribranchial cavities (Pl. 1, fig. 8, *c*). The whole structure arches over the nerve-cord, which it closely invests on three sides. The four figures *a*–*d* represent sections 9, 12, 14, and 16 out of a total series of 29. As only two sections are omitted between the first two figures, and only one section between each of the other pairs, the vesicle is seen to be of short extent. Fig. 8, *b* (Pl. 1), itself gives the impression of a somewhat deep bell-shaped cavity; but when the depth of the dorsal groove (fig. 8, *a*, *c*, Pl. 1) is discounted, the vesicle is seen to consist of two deep lateral pockets united by a slight median depression. In other words, the saddle-shape of the ultimate atrio-cloacal cavity is realized almost from the start.

It is this well-defined phase in the origin of the atrium that Pizon strangely misinterpreted as the development of a 'seconde vésicule sensorielle' (l. c., p. 65, fig. 87). Instead of realizing that the neural tube of an Ascidian larva is completely detached from the ectoderm before the sensory vesicle is differentiated, and that the latter is formed as a local expansion of the former, he described the neural tube at this stage as consisting of three separate elements, a posterior tube and two sensory vesicles, each connected independently with the dorsal groove, but none of them communicating inter se. Of the two vesicles one contained an 'eye-spot', and was completely closed; the other, which he expected to contain an otolith, was 'absolument vide', but still open to the exterior (Pizon, pp. 63–5, figs. 85–8). This strange account becomes intelligible when Pizon's figs. 85–8 are compared in reverse order with our figs. 8, *a–d* (Pl. 1). He had plainly overlooked the nerve-cord in his figs. 86 and 87, and had mistaken the atrial involution in fig. 87 for an imaginary (and quite unwarrantable) second sensory vesicle.

Moreover, Pizon's erroneous account of the origin of the peribranchial cavities from the pharynx is easily explained when the peculiar intricacies of the larval cavities have become familiar after prolonged study. His evidence is limited to four consecutive sections through a single larva of Botryllus violaceus represented by his figs. 66–9 (Pl. v). Pizon describes this larva as 'très jeune', but the fully constituted, thin-walled, cerebral vesicle, the elaborately folded pharynx, and the deep indentation of its dorsal wall by the swollen brain, all indicate a very advanced stage of development, equivalent to our Stage IV at least (cf. fig. 9, *d*, Pl. 1), and possibly to Stage V. His sections are described as 'proceeding from the posterior to the anterior part' of the larva, but it is easy to see that they proceed in just the opposite direction; for the visceral ganglion lies behind the otolith, and here it is present in the last section, the otolith in the second and third, and the membranous wall of the sensory vesicle alone in the first. If the sections proceeded as Pizon states, the nerve-cord ought to succeed the visceral ganglion in passing from the fourth to the first section; but it is the foremost part of the cerebral vesicle, and not the visceral cord, which is present in the first two sections.

The sections are also stated to be 'à peu près perpendiculaire à l'enroulement de la corde'. The series consisted of twenty-four sections, and the middle one appears as fig. 68. In this the tail is certainly cut through on either side of the embryo, but the notochord in each place is spindle-shaped, instead of round, and indicates a deviation of at least 45° from the perpendicular plane. Yet with sections as oblique as they could well be, Pizon treats them as if they were a series of transverse sections from behind forward, and 'à peu près perpendiculaires'. In point of fact they are more nearly longitudinal than transverse, and, at the same time, cut the front of the body before the rear, as we have seen. Having misinterpreted the orientation to this extent, it is hardly surprising to find that Pizon's identification of parts is seriously wrong. He identifies as peribranchial cavities two large diverticula of the pharynx 'qui s'étendent sur les faces latérales de la larve et sont symétriques' (p. 73). They are symmetrical and lateral only because of the obliquity of his sections, for one is dorsal and the other ventral to the equatorial plane. The first is quite clearly one of the three dorsal pockets of the pharynx caused in the later stages of development by the pressure of the brain on its roof (cf. our Text-figs. 3 and 10); the other on the postero-ventral side is the gastric outgrowth which will produce the intestinal loop.

Another diverticulum, shown in figs. 66 and 67, is claimed to establish the posterior endodermal origin of the dorsal (or hypophysial) tube. As a matter of fact the diverticulum in question is the anterior or oral pocket, shown in our Text-fig. 3, which is produced to meet the stomodaeal ingrowth from the anterior end of the dorsal groove shown in Pizon's own fig. 69 (*ov.*). If a diagonal were to be drawn across our Text-fig. 3 from the top left-hand corner through the anterior and left lateral pockets to a point a little in front of the base of the tail, an oblique section could be taken through the larva from this diagonal which would pass just in front of the dorsal groove, through the two pockets mentioned, the brain behind them, the gastric lobe and pericardium underneath, and through two regions of the tail, without touching the real atrium at all. It would correspond exactly with Pizon's figures. If a whole series were cut in this plane, the atrium would not appear until the razor had got well into the area of the dorsal groove. Pizon's last figure (29) only just begins to invade it, cutting obliquely across the oral diverticulum as his alleged 'organe vibratile'.

To resume our description, in the next stage (Stage IV) the lips of the dorsal groove have become so closely approximated throughout their length as to convert the groove into a virtual tube lying beneath the level of the general ectoderm (Pl. 3). Anteriorly (Pl. 3, fig. 15, *a*) the groove is still open and is seen to be produced inwards as a funnel (the stomodaeum) which meets a corresponding upgrowth from the anterior pharyngeal recess (it can hardly be called a pocket as yet), the oral diverticulum. Two sections farther back (Pl. 3, fig. 15, *b*) the groove is also open and the first sections of the neural tube appear. In fig. 15, *e* (Pl. 3), the cerebral vesicle with its contained otolith, now ventral in position, and the almost solid dorsal groove are cut through, and fig. 15, *g* (Pl. 3), shows the hinder end of the 'visceral ganglion' or larval brain. Above the narrow cavity of the neural tube in this figure can be seen the closed dorsal groove, lying as a rod between neural tube and ectoderm. From this point backwards the dorsal groove ceases to be distinguishable, for it merges internally into the bilobed atrio-cloacal vesicle, the front end of which is shown in fig. 15, *h* (Pl. 3, two sections behind *g*) as a dark solid mass occupying the site of the cerebral cavity in the previous figure. The atrial vesicle is temporarily asymmetrical in consequence of adjustment to the asymmetrical cerebral vesicle and ganglion in front and beneath it; it extends farther down on the right side of the figure (really left of the body) than on the other. Its cavity is recognizable in the two following figures (15, *j* and *k*, Pl. 3), and its hinder wall forms the dark bilobed asymmetrical mass above the nerve-cord in fig. 15, *l* (Pl. 3).

The future cloacal siphon is represented by the short cul-de-sac forming the apex of the atrial vesicle in fig. 15, *j* and *k* (Pl. 3). It still virtually opens into the hindmost end of the dorsal groove, which is represented in section by the spindle-shaped mass above the neural tube in fig. 15, *g* (Pl. 3), and by the small triangle on the top of the front wall of the vesicle in fig. 15, *h* (Pl. 3). Later stages show that when the cloaca regains communication with the outer world it does so in consequence of a reopening of the whole dorsal groove, but this does not occur before the tadpole is hatched and ready for liberation.

After Stage IV, with the exception of the point just mentioned, there is little to record except the gradual extension downwards of the peribranchial cavities on either side of the pharynx posteriorly, and perforation of the branchial walls by successive protostigmata. In an embryo ready to hatch there may be from six to eight protostigmata on each side, an increase on the number usual in Botryllus (Garstang, 1892; Grave and Woodbridge, 1924).

Our observations on the origin of the atrio-cloacal cavity of Botrylloides thus furnish a complete confirmation of the brief account given by Julin (1904) in the note mentioned in our preface. This account we may quote in full: ' Chez l'embryon des Botryllidae que j'ai étudiés (Botrylloides rubrum and Botryllus sulphureus) les ébauches des deux cavités péribranchiales procèdent d'une invagination unique et médiane de l'ectoderme, comme je l'ai constaté chez les Molgulidae et les Cynthiidae. En outre, cet orifice d'invagination se comporte comme je l'ai observé chez tous les autres Ascidiens dont la formation de l'orifice cloacal s'accomplit avant l'éclosion de la larve (Perophora, Distaplia, Molgulidae et Styelopsis), à savoir que ses lèvres s'accolent si intimement qu'elles paraissent soudées ' (l. c., p. 538, footnote).

As this accelerated mode of development is now known to occur in each of the three main families of the Stolidobranchia, and as the more typical mode of development by a pair of lateral invaginations is not known to occur in any member of this sub-order, we may regard the development of the atrium by a single median invagination as a general characteristic of the Stolidobranchiate Ascidians. Up to the present time it has not been observed in either of the other sub-orders of Ascidians (Phlebobranchia,[1] Haplobranchia). It is therefore interesting to note that the nearest approach to this method in other

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[1] Our use of Lahille's terms in preference to Seeliger's equivalents is defended in the paper which follows (p. 159).

Tunicata is apparently furnished by the Thaliacea, more particularly by Salpa and Doliolum. In Salpa, according to the conflicting accounts of Todaro and Salensky, the cloacal cavity develops either by a mid-dorsal invagination, which enlarges into a closed vesicle, and acquires its external opening by a second invagination at a later date (Todaro), or by some form of derivation from the pharynx (Salensky ; Korschelt and Heider, 1893, pp. 1342, 1353). The latter account has now lost the analogy of Botryllus in support of it, and can only be regarded as exceedingly improbable, unless the ' gill ' is really a diapharyngeal structure, analogous to that in the developing Ascidiozooids of Pyrosoma (Korschelt and Heider, l. c., p. 1332).

In Doliolum Uljanin's account (1884, pp. 54, 58, Taf. VI, fig. 14) of the origin of the cloaca from a mid-dorsal invagination in Doliolum mülleri, though confirmed by Salensky, must now be taken in relation with Neumann's more detailed observations on Doliolum denticulatum (1913, p. 108, Taf. XI, XII), in which the first signs of the peribranchial system are manifested as a pair of longitudinal grooves, or lines of proliferation, distinctly lateral in position (Taf. XII, fig. 9). These deepen into a pair of lateral pockets in front (cf. Fol., 1872, p. 450, footnote), diminishing in depth behind, and a process of approximation of the outer margins of the grooves keeps pace with their growth in depth. Thus at an early stage the cloacal area displays dorsally a Λ-shaped margin, complete in front like an Amphibian blastopore and dying away as its lips diverge behind (figs. 18–22). Some part of this appearance is doubtless due to Neumann's sections having been taken perpendicularly through peribranchial pockets which were directed obliquely forwards (Taf. XI, fig. 5), and the same remark is applicable to the earlier stage (fig. 3). But, while Neumann naturally emphasizes the difference between his observations and those of Uljanin, and the priority of the lateral peribranchial elements over the median cloacal aperture, it is clear that the method of formation of the cloaca in Doliolum is to a considerable extent intermediate between that shown by

Ciona and Clavelina on the one hand, and Botryllus and Molgula on the other. If the median origin of the cloaca in Salpa should be confirmed, the indications of a peculiarly close relationship between the Thaliacea and the Stolidobranchia in this respect, as in several others, would be obvious. The fact that Pyrosoma shows a much more distinctly paired origin of the atrium than either Salpa or Doliolum is doubtless indicative of its retention of a more primitive condition, in spite of the degeneracy of the oozooid.

One of the most remarkable peculiarities of Botryllid development, whatever its significance, is the differentiation and fate of the dorsal groove. Arising early (Stage II) before there is any indication of stomodaeum or cloacal rudiment, as a longitudinal plate of thickened ectoderm cells overlying the anterior part of the neural tube, it becomes converted into a groove, from which stomodaeum and cloacal rudiment are derived as outgrowths from its anterior and posterior extremities. Closing over them, it changes into an open or solid tube beneath the general surface of the ectoderm. In oblique sections its two extremities frequently appear to be withdrawn entirely from connexion with the ectoderm, owing to the slenderness of the intervening neck, but there is never any uncertainty as to its retention of connexion with the ectoderm in its central part (fig. 15, Pl. 3). In the tadpole ready for liberation it once more opens up throughout its full extent, exposing the oral and cloacal siphons, and after fixation and metamorphosis completely disappears.

Pizon (l. c., pp. 69, 70) recognized the groove as ultimately uniting ‘ les deux orifices branchiaux larvaires ’, but misinterpreted its origin as the anterior continuation of the neural tube : ‘ Dans la future région antérieure de la larve, le tube (neural) disparaît et se continue par un sillon épiblastique très accusé, dont les parois ont des cellules beaucoup plus volumineuses que le reste de l’ectoderme et qui sont en voie de prolifération active ’ (l. c., p. 63). It was this ‘ prolifération ’ which was actually producing the paired peribranchial sacs (cf. our fig. 8, *c*, Pl. 1, with his corresponding fig.

86); but he explained them as parts of a second sensory vesicle, remarking: ' Ces deux vésicules rappellent évidemment [!] celles que Kowalevsky et Kupffer ont décrites chez les Ascidies simples ' (p. 65).

In their admirable drawings of the larva of Botryllus schlosseri, Grave and Woodbridge (1924, figs. 1, 2, 10) show the dorsal groove filled with test-substance, thus blocking the openings of both oral and atrial siphons, and preventing them from functioning. It is difficult, however, to believe that the production of test-substance can be a primary reason for the differentiation of the groove, since the siphons are equally blocked in other Ascidian larvae without any such contrivance.

There seems to be no trace of any comparable structure in the development of the tailless larva of Anurella bleizi (Damas, 1902), or in the tailed larvae of Molgula echinosiphonica (Damas, l. c.) and Molgula citrina (Grave, 1926). On the other hand, in several adult Molgulids (e.g. Molgula oculata, Forbes, of British seas; Molgula conchata, Sluiter, South Africa) the two siphons are lodged in a depression which takes the form of a deep groove which can close completely over them; and the remarkable differentiation of the test in the Cionid Rhodosoma to form an operculum over the apertures, when retracted, is only a special development of the same device. It is therefore not improbable that the groove is a vestige of a similar contrivance in Botryllid ancestors. As a purely embryological phenomenon the conversion of the groove into oral and atrial siphons is curiously similar to the conversion of an elongated blastopore into mouth and anus.

§ 4. DEVELOPMENT OF THE NEURAL COMPLEX.

In the larvae of Botrylloides the nervous system consists at first (Stage I) of a tube, one cell thick, and of more or less the same dimensions throughout its length, running from the anterior end of the larva, along the dorsal region of the body and into the tail (Pl. 1).

Anteriorly, the neural canal communicates with the exterior by the neuropore (Pl. 1, fig. 1). Following this, Stage II, the neuropore closes, and the neural canal becomes detached from the ectoderm at its anterior extremity. Part of the canal near the front end begins to dilate. This enlargement of the cavity, to form the future sensory vesicle, takes place asymmetrically, so that the vesicle comes to protrude on the right of the nerve-cord. On its dorsal wall, one of the cells projects inside the cavity, and is the future otolith (Pl. 1, fig. 5).

The part of the neural canal anterior to the cerebral vesicle, which may be called the precerebral region, elongates slightly. It appears as a tube, one cell thick, with a central lumen, but posteriorly its communication with the cerebral vesicle becomes obliterated. Owing to folding of its walls, the tube-like appearance is lost almost at once, and the lumen becomes indistinct, so that, in transverse sections it seems to be a solid mass of cells (Pl. 2, fig. 10). In slightly older larvae, after it has constricted off the hypophysial duct (see later), it is definitely tubular, with a very clear lumen. It lies close against the endoderm and fuses with that layer by its blind extremity. No function except that of giving rise to the hypophysial duct can be assigned to this structure. It may be distinguished as the precerebral lobe (Pl. 1, fig. 9, *d*, *e*).

When the visceral ganglion has been formed on the ventral wall of the neural canal in the region of the cerebral vesicle, longitudinal sections through the medial region show the ganglion merging anteriorly into the precerebral lobe. In early stages these two are indistinguishable, but ultimately the cells of the lobe become clear and vesicular, showing no contents other than the nucleus, thus contrasting with the ganglionated part of the nerve-tube with its large nuclei and fibres (Pl. 2, fig. 11, *a*–*c*).

In the meantime the sensory vesicle has been developing. The otolith, after its appearance on the dorsal wall (Pl. 1, fig. 5), very soon comes to assume a ventral position (Stage III), in consequence of expansion and thinning of the dorsal wall of the vesicle and general enlargement of the cerebral cavity (Pl. 1, fig. 9, *d*). The wall of the vesicle is non-nervous, and in the older larvae the dorso-lateral region of the wall is reduced to a membrane. There is no separate eye in the Botryllidae, but the otolith has a complicated structure, according to Grave and Woodbridge (1924), which enables it to function both as a visual organ and as an organ of equilibrium.

Behind the sensory vesicle the neural tube is thickened ventrally to form the visceral ganglion (Stage III), from which an almost solid cord of nerve-fibres and cells extends backwards as the visceral nerve-cord (Pl. 1, fig. 8; Pl. 2, fig. 10, *a*), which is reduced in the tail to four cells only, as seen in transverse sections.

An important part of the neural complex has meanwhile arisen, the hypophysial duct. At an early stage of development, immediately before the dorsal wall of the cerebral vesicle thins out (Stage II), a strand of cells begins to be separated from its antero-dorsal wall, and the constriction is carried forwards along the precerebral region of the neural canal (Pl. 2, fig. 10). This rod of cells subsequently (Stage III) acquires a central lumen which is continuous with the cavity of the cerebral vesicle (Pl. 2, fig. 11, and Stage IV, Pl. 1, fig. 9; Pl. 3, fig. 15, *f*). The tube ends blindly in front, close behind the endodermal oral outgrowth, which is in contact with the still closed stomodaeal downgrowth from the dorsal groove; that is, just behind the future oral siphon (Pl. 2, fig. 11, *c*, *d*). It lies immediately underneath the dorsal groove already referred to.

The aperture of the hypophysial duct is soon carried backwards into the hinder part of the cerebral vesicle (Pl. 1, fig. 9, *f*), but eventually this opening closes up (Stage V), and the duct becomes detached from the cerebral vesicle altogether. It is then a tube, blind at both ends for a short time, abutting anteriorly against the oral endoderm, and posteriorly lying close to the front wall of the mid-atrium, where the right and left peribranchial diverticula are in contact with one another. As the visceral cord continues to run from the visceral ganglion beneath the cloaca into the tail, and encloses a virtual lumen, the effect of these changes is that the primary neural tube is completely separated, before the larval stage begins, into a larval portion, consisting of sensory vesicle, ganglion, and caudal nerve-cord, and an adult portion, consisting of the hypophysial tube alone, which ultimately gives rise to the adult ganglion and neural gland. This exceptional arrangement explains the absence of any trace of a ' rapheal duct ' behind the brain in adult Botryllids.

Although the dorsal groove lies directly above the rod of cells which differentiates to form the hypophysial duct, and the latter is pressed closely between the precerebral lobe and this groove, the latter takes no share in its formation, though this has been a very difficult point to decide, and sections are rather suggestive of the other possibility. It should be noticed, however, that the dorsal groove extends not merely along the anterior end of the larva, to which the hypophysial duct is restricted during its formation, but backwards directly above the nerve-cord, as far as the base of the tail (Pl. 2, fig. 10).

When the first protostigmata are pierced (Stage V) the hypophysial duct is no longer a blind tube, but has acquired an opening anteriorly into the pharynx, just behind the mouth. This opening becomes lined by ciliated cells, but we have been unable to determine their exact mode of origin. The walls both of the tube and the ciliated funnel are made up of small, rounded, and entirely non-nervous cells (Pl. 2, figs. 12, 13).

At this stage also, the degeneration of the larval nervous system begins, and the part which disappears first is the precerebral lobe (Stage VI). Phagocytes can be seen in its place. When the free-swimming tadpole stage is reached (Stage VII) the ventral wall of the hypophysial duct begins to proliferate, and the cells so produced form a little mass lying underneath the tube (Pl. 2, fig. 13). This is the beginning of the adult brain. It remains connected with the duct posteriorly by a thin strand of cells, even when fully differentiated (Pl. 2, fig. 12).

The hypophysial duct has two very distinct parts at this stage (Stage VII), the anterior ciliated funnel, and a tube, which is not ciliated. This tube is dilated somewhat towards its anterior end, but posteriorly it becomes more or less solid, and in the adult this portion is continuous with the hinder end of the ganglion, which it has formed at an earlier period.

SUMMARY.

The development of Botrylloides adds a new feature to the already complicated structure of the neural canal in Tunicata. The anterior pre-sensory region of the canal was already known in Ciona, Clavelina, and Distaplia to become converted into part of the so-called hypophysial duct, which terminates in front in the ciliated funnel and gives rise to the subneural gland and adult brain. Here, in Botrylloides, the same pre-sensory region is much more pronounced and becomes longitudinally differentiated into two parts, lying one above the other—a large ventral precerebral lobe, which disappears entirely before the tadpole stage is reached, and a slender dorsal precerebral duct, which persists, and is comparable in all essential respects with the hypophysial duct of other Ascidians. The duct in Botryllids communicates with the oral region of the pharynx by a ciliated funnel and gives rise to the brain by proliferation from its ventral wall behind. It fails to produce a special neural gland, but as its own walls assume a glandular character in the adult stage, the difference in this respect is insignificant. Its position above the adult brain is shared with most Stolidobranchiate Ascidians, and in these the neural gland, when present, is developed above the brain, instead of beneath it. The one Botryllid feature which demands special explanation, therefore, is the peculiar development and fate of the large, transitory precerebral lobe in front of the cerebral vesicle.

III. SIGNIFICANCE OF THE PRECEREBRAL LOBE (W. G.).

§ 1. After the earliest period of differentiation the topographical relations of the precerebral lobe to the incipient hypophysial duct are nearly those of the subneural gland to the hypophysial duct in a Ciona or Clavelina. The first idea that presents itself, therefore, is to regard the lobe as a precocious manifestation of the subneural gland, which, as such, would otherwise in Botryllids be absent. On further reflection, however, this interpretation, though attractively simple, seems untenable for the following reasons :

(1) The subneural gland of Ciona, &c., is the same gland as the supraneural gland in Cynthia, Styelopsis, and most species of Molgula, the adneural gland of Molgula roscovita, the subneural gland of Molgula citrina (vide Grave). It may change its position as a dorsal or ventral outgrowth in closely related species of Cynthia and Molgula, and may be represented, without any outgrowth at all, simply by the glandular wall of the hypophysial duct itself (Julinia). Its equivalent is therefore already present in Botryllus in the form of one or more dilatations of the hypophysial duct above the brain (E. C. Herdman, 1924, figs. 13–15, after Metcalfe, 1900).

(2) Under these circumstances any phylogenetic change in the position of the gland, e.g. from ventral to dorsal, would be accomplished directly. It is impossible to believe that a ventral representative of the gland must first appear in the embryo, and then disappear, before a dorsal gland could come into being.

(3) The subneural gland is a secondary outgrowth of the hypophysial duct, but in Botrylloides the duct is a product of the precerebral lobe.

(4) The lobe actually opens into the pharynx independently of the duct.

§ 2. A second theory of the precerebral lobe may be based on the fact that up to the time of its disappearance it retains continuity with the larval ganglion behind, while the hypophysial duct has similar relations to the adult ganglion. The originally single cavity of the neural tube becomes in fact divided into larval and adult moieties. Seeliger in particular (in Bronn, pp. 791–4) regarded the separation of sensory vesicle and hypophysial tube as a process of longitudinal fission of the primary neural canal. On the current theory that Ascidian larvae represent the ancestors from which adult Ascidians have been derived by degeneration, part of the nervous system of the larva ought to persist as that of the adult and is commonly stated so to do (cf. Herdman, 1904, p. 61; Sedgwick, 1909, p. 23). In point of fact not a cell or fibre of the larval nervous system is known to persist after the metamorphosis, and there is a complete breach of continuity between the two (cf. Van Beneden et Julin, 1887, p. 353). Under these circumstances, the only way in which the larval and adult nervous systems can be brought into proper theoretical relation to one another is to assume that originally the two were combined. If now we compare the development of these parts in Botrylloides with that in Ciona and Clavelina as described by Willey (1893), and in Distaplia as described by Hjort (1896) and Salensky (1893), we see that in all cases the division of the primary neural tube takes place in such a way that the larval sensory vesicle lies on the right, the hypophysial tube on the left. Assigning the larval ganglion to the former and the adult ganglion to the latter, we may regard these two elements as antimeres of an originally bilobed, or bilaterally symmetrical, structure; and we may assume that gradually the larva took over the right and the adult the left antimere as the basis of their respective nervous systems.

This is certainly a peculiar thing to have happened, but it is not entirely without analogies. Larval butterflies take over the mandibles, and their imagines the maxillae. In various Crustacea there is an allelochronic distribution of the branches of appendages, so that only exopodites develop in the larval phase, and endopodites only in the adult. The spinal nerves of one side of the body innervate both sides of the oral hood in Amphioxus.

The peculiarity of Botrylloides on such a theory would then be reducible to this, that, while in most Ascidians the precerebral region remains undivided, in Botryllids it shares in the differentiation between larval and adult moieties, thus suggesting that the precerebral organs themselves were originally paired and symmetrical.

It is certainly remarkable that the precerebral lobe in Botrylloides undergoes a course of development which runs parallel in many respects to that of the hypophysial tube in other Ascidians especially Clavelina. Each includes the anterior undivided neuroporal extremity, enlarges, and fuses anteriorly with the oral region of the endodermal pharynx, but whereas the hypophysial tube of Clavelina proceeds to develop a ciliated funnel in front, and a neural gland behind, the precerebral lobe splits off a dorsal section which does the same things, while the lobe itself atrophies and disappears without leaving a trace (cf. our fig. 9, Pl. 1, for Botrylloides with fig. 14, Pl. 2, for Clavelina).

The only objections to this antimere theory, but they seem to be fatal, are that it rests on a basis which cannot easily be granted and is inconsistent with some plain and simple facts. The asymmetry of the sensory vesicle is a secondary, not a primary, feature. By its development it is clearly a median structure, bearing a series of primitively median sense-organs (Willey, l. c., figs. 1, 3). If its ultimate twist to the right gives it a claim to be regarded as an antimere to the adult brain, what are we to do with the Appendicularians, in which the orientation is exactly reversed, viz. otocyst to the left and ciliated funnel to the right? Moreover, the division of the presensory region of the neural tube in Botrylloides into lobe and duct is horizontal and not vertical. We can regard these structures as successive manifestations of the same phenomenon, or as repetitions of the same process, in the median plane, but we can scarcely regard them as antimeric without mental torsion.

It may be added that while this hypothesis also in the last resort breaks down, it seems to carry with it the untenability of the current theory of Ascidian metamorphosis. This aspect of the matter will be dealt with in the paper which follows.

§ 3. Having failed to find anything strictly comparable with the precerebral lobe in the well-confirmed accounts of the development of Ciona and its relatives, we turn to the forms mentioned in our preface as having probably a more intimate degree of relationship. Unfortunately among the Stolidobranchiate Ascidians, apart from Botrylloides, only one species has been studied in detail from the developmental standpoint, and that is one of the small Molgulids with a degenerate tailless embryo, Anurella bleizi (Damas, 1902). The development of an anomalous form of this kind presents many points of interest, but cannot be assumed to be representative of the course of events in the more normal members of its family.

The most remarkable feature in the development of the nervous system which Damas claims to have shown in Anurella is the origin of the hypophysial tube from a common 'bucco-hypophysial' rudiment, i.e. by an involution of ectoderm which yields both stomodaeum and hypophysial tube. If this claim is well founded, it means that the hypophysial tube in Ascidians arises in two different ways, (*a*) from the neural tube in Ciona, Clavelina, Distaplia, and Botrylloides, and (*b*) from stomodaeal ectoderm in Molgulids. After our demonstration of the invalidity of Pizon's claims in Botryllus, we may be somewhat unduly prejudiced in favour of uniformity; but a careful examination of Damas's paper has convinced us that his evidence is inconclusive and that the problem needs renewed investigation.

Damas's investigation of this point begins with transverse sections of a young embryo 'après la fermeture complète du tube médullaire' (Pl. xxvi, figs. 1–9), but no statement is made as to the stage of development at which the neuropore actually closes. In view of the anomalous mode by which the neural tube is established in Anurella, nothing can be assumed on such a crucial point. So far as evidence goes, it appears perfectly possible that fig. 4, which Damas claims to be 'l'ébauche buccale', is in reality a section through the neuropore. If so, the separate 'bucco-hypophysial' invagination has no existence. To support this opinion, we may point to the fact that at this stage, the earliest examined, the alleged 'ébauche' of stomodaeum and hypophysis is already in such intimate contact with the cerebral vesicle behind that Damas remarks: 'La limite entre l'ébauche buccale et le cerveau est difficile à tracer avec certitude' (p. 632).

In brief we suggest that Damas's fig. 4 corresponds with our fig. 4 (Pl. 1), that his 'ébauche bucco-hypophysaire' is our 'precerebral' region of the neural tube, and that his fig. 6, which is claimed to represent the meeting of hypophysis and cerebral vesicle, corresponds approximately with our fig. 10 (Pl. 2), which shows the precerebral lobe merging into the front of the sensory vesicle, and causing a pronounced thickening of the cerebral wall on its ventral side.

If this criticism is correct, it is to be noted that Anurella agrees with Botrylloides in showing the persistence of a considerable precerebral region of the neural canal, unlike the condition in Ciona, Clavelina, &c., while it differs from Botrylloides in undergoing a direct conversion of the precerebral region into the hypophysial tube. This, indeed, seems to be a general Molgulid character, irrespective of the nature of the larva. There is little direct evidence bearing on this question, since Damas figures only one section (Pl. xxviii, fig. 1) of the larva of Molgula echinosiphonica at a sufficiently early stage through the precerebral region. Nevertheless, his figures establish a related point of even greater significance, viz. that the cerebral cavity itself is not divided. Julin's figures of Lithonephria eugyranda (copied in Seeliger, l. c., fig. 190) show the same thing. In neither case is there any trace of a hypophysial canal by the side of the cerebral cavity in sections through the plane of the otolith. The same feature is also shown in Grave's recent admirable figures (1926) of the free-swimming larva of Molgula citrina, which represent the hypophysial duct opening into the front, not the rear, portion of the cerebral vesicle (cf. Damas, l. c., figs. 12–15). We can therefore assert that the entire neural canal in Molgulids retains its primitive undivided condition.

Thus Ascidians present a series of stages in the differentiation of the neural tube:

(*a*) In Molgulids the whole neural tube remains undivided, whether a tailed larva is developed or not. There is no special sensory vesicle, but the primary cerebral cavity, containing an otolith but no eye, functions as one. A well-developed precerebral region persists as the hypophysial duct in the anurous, and probably in all forms.

(*b*) In Ciona, Clavelina, &c., the cerebral cavity is divided by constriction from it of a special sensory vesicle. The precerebral region remains undivided, but temporarily shrinks almost out of recognition owing to precocious development of the sensory vesicle (containing otolith and eye), and then grows out again as the hypophysial duct.

(*c*) In Botryllids the precerebral region is well developed and persistent, but, along with the cerebral region, is divided into larval and adult moieties. The sensory vesicle contains a single sense-organ of complex structure, functioning both as otolith and eye (Grave and Woodbridge, 1924).

The question then arises, which of these conditions is primitive? Is the series (*a*), (*b*), (*c*) a progressive one; or are (*a*) and (*c*) derivable from (*b*) by simplification in the first case, and further differentiation in the second; or is (*c*) primitive, and (*a*) and (*b*) derivatives from it by simplification on the one hand, and simplification combined with further differentiation on the other?

If we turn to the Thaliacea for some independent light on these questions, the bare facts of development might seem to support the first solution. For both in Pyrosoma, Salpa, and Doliolum the whole neuro-hypophysial complex of the oozooid develops from a single undivided neural tube or vesicle, in which a precerebral region is differentiated at an early stage and is directly transformed into the hypophysial duct (cf. Korschelt and Heider, figs. 781, 784 for Pyrosoma; figs. 801–11 for Salpa; figs. 766–8 for Doliolum. Also Neumann, 1913).

[Incidentally these cases establish a point of some theoretical importance, viz. the complete absence of any stomodaeal element in the formation of the ciliated funnel, for this is developed and opens into the endodermal pharynx before the mouth itself is pierced. The same features and result have already been described for Clavelina by Seeliger (in Bronn, p. 799), but Huntsman, in his extraordinary theory of the Ascidian mouth (1907), has taken no account of these cases. We have ourselves confirmed the accuracy of Seeliger's statements (see our Pl. 2, fig. 14, *a–d*). That a stomodaeal element is not necessary to the formation of the ciliated funnel is independently established by the peculiar relations in Phallusia mammillata (Julin, 1881, Pl. xiv; Seeliger in Bronn, p. 306), the duct of which is provided with secondary ducts terminating in funnels resembling the primary one, but opening into the cloaca. It may even possess numerous cloacal funnels without a stomodaeal one at all (Herdman, 1883).]

Unfortunately the directness of development in Thaliacea, apart from other factors, is subject to the same qualifications of interpretation as that of Anurella, viz. the possibility of a secondary simplification of the development in consequence of suppression of the larval form, which presumably possessed its special sense-organs. That the organization of the degraded larva of Doliolum originally resembled that of Ascidian larvae more closely than to-day is fairly clear from the similar disposition of its caudal muscles and certain other features, but who can say how far this resemblance extended? Did it possess an otolith, an eye, or both, or neither?

In this connexion one peculiarity may ultimately yield some light, the extraordinary fact that while Appendicularians possess a cerebral otolith on the left side, Doliolum possesses a similar left-sided otolith, but outside the neural tube altogether. It is difficult to believe that these two are not homologous structures, especially as an otolith is lacking in the bud-generations. At any rate the correspondence suggests the possibility that the larva of Doliolum originally possessed a cerebral otolith which has migrated outside the neural field and been retained in adaptation to the retention of pelagic habits. Neumann has shown (1913) that the orifice, which Uljanin (1884) regarded as marking a secondary invagination of endoderm, is in reality the neuropore, which remains open much longer than in normal sessile Ascidians. This prolonged retention of the neuropore is the consequence of a general retardation in the forward growth of the neural folds (Neumann, pp. 102, 105), as can plainly be verified by inspection of the successive positions of the neuropore in Neumann's figs. 1, 3, and 5 (Taf. XI).

Yet in the earliest of these stages the notochord and caudal muscle-cells are already in full process of degeneration. This delay in the closure of the neural folds, during a period of active growth, may conceivably have resulted in the escape of the otolith-forming cell outside its original boundaries. Uljanin (l. c., p. 56) has described the formation of the otolith as the product of a single cell, just as in the case of Ascidian larvae. In the primitive Doliolum mülleri it remains freely exposed; in the more advanced Doliolum denticulatum, &c., it is secondarily enclosed in a cellular vesicle formed by invagination and overgrowth of ectoderm. The absence of an otolith in Salpa, like its absence in the buds of Doliolum, furnishes a strong point in support of this hypothesis, since the habits and requirements of the adult stage are approximately alike in Doliolum and Salpa, but the larval stage of Salpa has been more completely suppressed.

If there is any force in this argument, it follows that the original larvae of Thaliacea may have been equipped with sense-organs at least as elaborate as those of Botryllus and Ciona, and that the cerebral development in Thaliacea may have been secondarily simplified, in consequence of their loss.

§ 4. The effect of these various considerations is to show that the group of organs we have been considering, consisting of ciliated funnel, duct, and neural gland, is not only characteristic of Tunicates generally, but arises in essentially the same way throughout the group by transformation of the anterior end of the embryonic neural tube. When the larva is suppressed, as in Anurella and the Thaliacea, the transformed region lies in front of the adult brain, and the whole course of development is direct and symmetrical. There is a clear division of the neural tube into an anterior cilio-glandular, non-nervous, region and a posterior nervous region. No sense-organs are involved. On the other hand, when development takes place through a larval form, this directness and symmetry are disturbed. It is as if some alien factor had been introduced, foreign altogether to the permanent nature of the adult Tunicate, whether sessile or free-swimming. One is reminded of the corresponding asymmetry of the larval Amphioxus, for no one doubts that the asymmetrical features in that case are capable of some special explanation, which would leave the primitive Protochordate bilaterally symmetrical in almost every respect. In the case of Ascidians this disturbing element is in some way associated with the larval sense-organs. If it can be shown to be caused by them, then the direct development of Anurella and the Thaliacea may be regarded as a reversion towards a primitive condition in consequence of the removal of the disturbing factor. It should be noted that we assume no tendency of developmental stages to recapitulate the stages of adult ancestry, but simply that a certain constitution is inherited which under normal conditions will express itself by a certain course of development, and that modifying factors, genetic or epigenetic, have specific results.

We have seen that in Molgulids there is no formation of a special sensory vesicle, even in the tailed larva, so that the problem in that family is reduced to its simplest terms. The tailless embryo of Anurella develops no cerebral sense-organs, and its neural tube differentiates into precerebral (or hypophysial) and cerebral regions with but the slightest indications of asymmetry. The wall of the cerebral vesicle remains thick and cellular all over, and is merely a little thicker on the left than on the right (Damas, 1902, Pl. xxvi, fig. 7). The cerebral wall also remains intact during the stage corresponding to the metamorphosis of the tailed forms, the only signs of which are to be seen in the casting-out and disintegration of a certain number of individual cells, which presumably are the material for a larval ganglion no longer required (l. c., figs. 13, 14).

Among what we may call ‘normal’ Molgulids, developing through a larval phase, there are some which more nearly approach the Anurella condition than others. These possess short-tailed larvae and Julin's Lithonephria eugyranda (=Ctenicella lanceplaini, Lac.-Duth.) is an example (see Seeliger, l. c., p. 1171), but there is no complete account of its development. The only point which we can extract from Julin's figures (Seeliger, l. c., fig. 190) is that, in spite of its short tail, the larva possesses a large median ventral otolith in a capacious cerebral vesicle, which, as in other Ascidians, is thin-walled on its upper and right (or external) sides.

The only other Molgulids of whose development any relevant points are known, seem all to possess long-tailed larvae, viz. Molgula echinosiphonica, Molgula citrina, and Molgula ampulloides. The two former may be associated, the only known difference being that in echinosiphonica the larvae complete their metamorphosis in the parental atrium, while in citrina they enjoy a brief free-swimming career of about 15–20 minutes' duration (see Grave, l. c.). The otolith is very large (16μ in Molgula echinosiphonica, according to Damas's figures, 1902), the cerebral vesicle capacious and thin-walled except on the left side, and the opening of the hypophysial tube into the front of the vesicle is distinctly left-sided and asymmetrical (Grave, l. c.).

Molgula ampulloides (De Selys and Damas, 1900) is apparently unique in its retention of the larval otolith throughout life in the wall of the neural gland, which here lies above the brain. The persistence of the otolith in this position, under conditions rendering it quite useless, affords strong, if not conclusive, evidence that in this case, as in that of Anurella, the wall of the cerebral vesicle remains intact during the process of metamorphosis. It must simply shrink to form the blind end of the hypophysial duct, even if, as is probable, it rejects a number of over-specialized cells in the process. The otolith is 10μ in diameter, i. e. not so large as in the previous case, but too large to pass down the hypophysial duct. Seeliger, in his discussion of this case (l. c., p. 851), strangely rejected this simple explanation by assuming 'dass—so wie bei allen anderen Ascidienlarven—auch hier eine vollständige Auflösung der Sinnesblase in Zellen und Zellstücke erfolgte', and by 'Sinnesblase' he had already told us that he meant, not the primary undivided neural cavity, but 'das rechtseitige, die Sinnesorgane führende Gebilde . . . das durch die Längsspaltung des Medullarrohrres zur Sonderung gelangt' (l. c., p. 794). Although Damas's and Julin's figures were before him, he seems not to have realized that the larvae of Molgulids differ from 'allen anderen Ascidienlarven' precisely in their failure to separate a special 'sensory vesicle' from the cerebral cavity, and it is this part only which undergoes histolysis at the metamorphosis of ordinary Ascidians.

As a matter of fact Damas's figures of Molgula echinosiphonica show that the sensory side of the cerebral vesicle breaks down exactly like a typical sensory vesicle (cf. Damas, 1902, figs. 14, 15), and the otolith consequently falls with other fragments into the haemocoele. The origin of the adult brain seems uncertain. Grave represents it in citrina as a dorsal outgrowth of the hypophysial tube in front of the larval cerebral vesicle (l. c., fig. 5), but there is no clear indication of such an origin in Damas's sections of a metamorphosing echinosiphonica (l. c., Pl. xxviii, figs. 12–14). There are probably many variations among Molgulids in the details of the process of reconstitution, which may account for the variable arrangement of the rapheal ducts and 'cordon visceral' (Metcalfe, 1900; Seeliger, l. c., pp. 295, 305).

This survey of somewhat isolated scraps of evidence seems to be sufficiently coherent to justify two opinions, (1) that the simplicity of development of these parts in Molgulids is the result of a secondary reduction of the larval element in the ontogeny, and (2) that the simplification has proceeded by stages directly connected with reduction of the larval sense-organs. These stages are as follows:

A. Tailed Larvae.

(*a*) A large thin-walled expansion of the cerebral vesicle, enclosing a large otolith, which arises dorsally but rotates down the right side to a mid-ventral position within it, and in the sagittal plane of the larva (Grave, l. c.). The continuation of the neural axis along the hypophysial duct is pushed to the left by this process. The right wall undergoes disintegration with loss of the otolith at the metamorphosis, e.g. Molgula echinosiphonica, citrina. (If Grave is right in identifying with this species Kingsley's 'Molgula manhattensis', the otolith is retained in situ for at least a month after atrophy of the tail. Cf. Kingsley, 1882, pp. 447–8, fig. 19.)

(*b*) As above, but otolith smaller. Cerebral wall remains intact at the metamorphosis, and retains the otolith in the wall of the neural gland, e.g. Molgula ampulloides.

B. Tailless embryos.

(*c*) Neural axis remains straight throughout development, the cerebral cavity is but slightly enlarged, and its wall remains intact. No otolith is developed, e.g. Anurella bleizi.

The short-tailed larvae of Lithonephria (=Ctenicella) may be expected to fall into the series between (*b*) and (*c*), but details are wanting as to the metamorphosis.

If now we compare the larval organization of Molgulids with that of Botryllids the difference between them is seen to be out of all proportion to the slight difference apparent in the sense-organs themselves. The pigmented otolith of Botryllus functions also as an eye, and has some corresponding complications of its finer structure (Grave and Woodbridge, 1924), but it occupies no more space than the otolith of Molgula, which, though similarly pigmented, betrays no visual functions at all (Grave, 1926). Yet in Botryllus, unlike Molgula, the otolith is isolated in a secondary sensory vesicle completely cut off from the neural tube, and projecting to the right in a conspicuously asymmetrical manner.

The clue to their evolution is provided by extending the comparison to the Cionid Ascidians. In these a sensory vesicle of large size is present on the right side in all cases, but includes a multicellular eye behind as well as a unicellular otolith in front (figures in Carrière, 1885, fig. 67). The cell-material as well as the time-requirements for the development of this vesicle are necessarily twice as great as in either of the other cases, so that, from the outset of development, the whole material of the neural tube anteriorly is drawn upon for the purpose. In the earliest stage, as shown by Willey (1893, figs. 1, 3), the region of the neural tube in front of the otolith cell is as long as the cerebral dilatation, but this pre-sensory, or precerebral, region is rapidly absorbed by early expansion of the cerebral wall (l. c., fig. 5), and it is not until the sense-organs have been constituted that the precerebral region is able once more to assert itself by a secondary process of outgrowth.

Suppose now that at some past period such a condition represented the high-water mark of evolution of the Tunicate larva, and that in the course of adaptation of Ascidians to different conditions the larval life in certain strains became reduced, as it certainly has in that of the Molgulids. In spite of numerous secondary re-adaptations, these Ascidians, from their entire organization, appear to be essentially and primarily inhabitants of spacious sand-flats in which they live immersed, loosely anchored by their coating of adhesive hairs. Suppose also that larval phototropism ceased in these cases to be of value, or even became definitely disadvantageous by causing the larvae to be carried away from the adult terrain, then the larval multicellular eye would sooner or later disappear, and the condition of the Molgulid larval brain would be established. What would be the developmental consequences? The draught on the neural epithelium would be considerably reduced, and the precerebral region would persist as a definite anterior region of the neural tube, which would develop directly into the hypophysial organs. Moreover, as the otolith had become the sole larval sense-organ, it could obviously serve orientation better in a median than in an eccentric position, and the separation of a special asymmetrical sensory vesicle could be advantageously eliminated. In both respects the Molgulid condition would be attained, and by stages which are consistent with the developmental variations in the different types. But the process is not reversible. The origin of the cerebral eye from a thin-walled cerebral vesicle of the Molgulid type is unthinkable.

The Botryllid case, which we are attempting to work up to, is clarified by these considerations, for there is reason to suspect that the so-called otolith owes its complexity not to further differentiation of the Molgulid structure but to an amalgamation of the Cionid otolith and eye. Indeed, it will appear from the sequel that in all probability the Botryllid and Molgulid conditions represent two stages of retrograde larval evolution from the Cionid. The eccentricity of the sensory vesicle in Cionids and Botryllids is clearly not due to any requirements of the otolith itself, but is related in some way to the visual function. The eye in fact is part of a phototropic mechanism for maintaining the larva in the upper waters by which it can be widely distributed. For this purpose it is not necessary that the larva should be always swimming; on the contrary it is small and light for the purpose of drifting, and the life of most phototropic larvae consists of alternating periods of passive drift, during which they slowly fall, and of active vertical locomotion. The eye is directed forwards so that the larval movements are stimulated towards the source of light above. But when the larva is drifting and falling the eye is directed downwards, and without some special provision would never receive its upward stimulant. So the otolith is fixed eccentrically, not over the centre of gravity as in a Molgulid, and the axis of the eye is set obliquely. As soon as the larva, when falling, awakes to the call of its eccentric otolith, it is compelled to rotate, the eye consequently traverses zones of different degrees of light, and the larva is led spirally upwards to the surface. 'Alles ist angepasst,' as morphologists too often forget. The story is probably not quite so simple as we have here imagined it, but experiment is not likely to alter the main proposition that the eccentricity of the phototropic apparatus is adaptive to larval requirements.

How far the behaviour of a Botryllus larva resembles and differs from that of an Ascidia or Ciona[1] has not, so far as we are aware, been yet determined, nor is there any accurate information on the duration of the free-swimming phase of the latter forms to compare with Grave and Woodbridge's excellent accounts of Botryllus and Molgula (l. c.). There is great variability, especially under laboratory conditions, and Ascidian larvae are not usually identified in plankton collections. But there is no doubt that the pelagic career of a Phallusia or Ascidia is usually a matter of days, while in Botryllus it is one of hours, and in Molgula of minutes or seconds. The interesting result follows from Grave and Woodbridge's experiments that in Botryllus the duration of the free-swimming period is probably determined by structural changes which take place in the 'otolith' itself or its nerve-connexions, since the larval reactions change from positive to negative phototropism shortly before metamorphosis.

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[1] Castle states (1896, p. 212) that the larval stage of Ciona may last 'from 24 hrs. to several days', but his remarks on its negative phototropism must be read in the light of Grave's corrections on this point in Amaroecium (1920). The average duration of the larval period in the

From the striking account given by the American authors, whose workmanship inspires every confidence, the otolith[1] of Botryllus has a complexity of structure which, as already remarked, suggests a combination of the Cionid otolith and eye, although there are no adequate observations on the development that bear upon the point. The fact that incidental observations up to the present time have merely revealed the origin of the otolith itself from a single cell does not preclude the possibility; and Grave's detection of two nuclei in the much simpler otolith of Molgula is highly significant (1926, p. 464). Moreover, the histological details given by Grave and Woodbridge (l. c., figs. I, J) appear to demand the incorporation of several elements. The resulting structure is as if a much simplified Cionid eye had been superimposed on the top of an ordinary otolith hollowed out for its reception. It should be remembered that according to Willey (l. c., figs. 1, 3) the visual cells at first form a single series immediately behind the single otolith cell in the mid-

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latter case is given by Grave as between 50 and 65 minutes, i.e. about the same as in Botryllus. It is therefore noteworthy that the larval eye, while typically Cionid, has apparently undergone a reduction of elements (1921).

[1] The word 'photolith' might appropriately be used to distinguish the Botryllid organ.

dorsal line, so that the possibility of a combination of these elements, in the building of the Botryllid organ, cannot be ruled out simply by the normal separation of otolith and eye in the fully developed Cionid condition.

If this hypothesis should receive the necessary confirmation, a satisfactory explanation of the peculiar mode of development of the Botryllid hypophysis would seem to be possible. For, assuming an origin from a Cionid condition of all the larval parts concerned, the amalgamation of the rudiments of otolith and eye would reduce the spatial and material requirements to the Molgulid level, so that the precerebral region might be expected to persist as in the latter case. On the other hand, retention of the phototropic mechanism, even on a reduced scale, would involve persistence of the eccentric position of the otolith and its asymmetrical capsule, so that, unlike the case of the Molgulids, a separation of the hypophysial canal from the general neural cavity would still be necessary. In Ciona this separation proceeds to the foremost extremity of the neural cavity. As there is no sharp boundary in a young Botryllid embryo between precerebral and cerebral regions, the persistence of the process of separation would presumably involve its extension along the precerebral lobe as well.

From a developmental mechanical standpoint, therefore, the division of the precerebral region of the neural canal in Botrylloides may be, in a sense, an embryological accident, due to the persistence of a process of septation which began phyletically under one set of conditions and was continued under another. It throws a distinct light on the phyletic problem, but without any assumption that the developmental sequence recapitulates the condition of parts in a succession of adult ancestors. If our interpretation is correct—and it admittedly depends on a contingency which has still to be verified—it renders intelligible the peculiar parallelism in the developmental history of the transitory and permanent moieties of the precerebral region, but does not require us to believe that any Tunicate ancestor ever possessed two hypophysial tubes one above the other.

With these considerations we have proceeded as far as direct comparison with other Tunicates enables us to go. It is fairly clear that a considerable development of the pre-sensory region of the neural canal and its glandular modification was a primitive feature of the class, and that these features distinguish the Tunicata both from Amphioxus and the Vertebrata. Their significance demands a wider survey of facts than would be appropriate in a descriptive paper, as well as a freer and more speculative treatment of the phylogenetic question. In the meantime the desirability of a wider knowledge of the development of Stolidobranchiates is clearly indicated, together with any and every contribution that can be made to our knowledge of the embryology of Doliolids, especially Anchinia. It is unfortunate that Neumann, in his valuable report on Doliolum, adopted a purely diagrammatic mode of representing his sections of the various stages of development.

BIBLIOGRAPHY.

Arnbäck-Christie-Linde, Augusta (1923).—" Reproductive Organs of Metrocarpa n.gen. and Botryllus. Northern and Arctic Inv., &c. IX. Tunicata. 2. Botryllidae ", ' Kongl. Svenska Vet. Handl.', lxiii. 9.

Carrière, J. (1885).—' Die Sehorgane der Thiere.'

Castle, W. E. (1896).—"The early Embryology of Ciona", 'Bull. Mus. Comp. Zool., Harvard', xxvii.

Damas, D. (1902).—" Recherches sur le Développement des Molgules ", ' Arch. de Biol.', xviii.

De Selys Longchamps et Damas, D. (1900).—" Recherches sur le Développement post-embryonnaire et l'Anat. de Molgula ampulloides ", ibid., xvii.

Delsman, H. C. (1910).—" Beitr. z. Entwickl. v. Oikopleura ", ' Verh. Rijksinst. Onderzoek ter See ', iii.

—— (1912).—" Weitere Beob.", ibid., xii.

Garstang, W. (1892).—" On the Development of the Stigmata in Ascidians ", ' Proc. Roy. Soc.', li.

—— (1895).—" Budding in Tunicata ", ' Science Progress '.

—— (1895 b).—" Outlines of a new Classification of the Tunicata ", ' Rep. Brit. Assoc. Ipswich '.

Garstang, S. L., and W. (1926).—" On the Development of Botrylloides and the Ancestry of Vertebrates (Preliminary Note) ", ' Proc. Leeds Phil. Soc.', i.

Grave, C. (1920).—" Amaroucium constellatum. I. Activities and Reactions of the Larva ", ' Journ. Exp. Zool.', xxx.

—— (1921).—" Ditto. Structure of the Larva ", 'Journ. Morph.', xxvi.

—— (1926).—" Molgula citrina. Activities and Structure of the Free-swimming Larva ", ' Journ. Morph.', xlii.

Grave, C., and Woodbridge, H. (1924).—" Botryllus schlosseri : the Behaviour and Morphology of the Free-swimming Larva ", ibid., xxxix.

Herdman, E. C. (1904).—" Botryllus ", ' L.M.B.C. Memoirs ', xxvi.

Herdman, W. A. (1883).—" On the Homology of the Neural Gland in the Tunicata with the Hypophysis Cerebri ", ' Proc. Roy. Soc. Edin.', xii.

—— (1904).—" Tunicata ", ' Cambridge Natural History '.

Hjort, J. (1896).—' Germ-layer Studies based upon the Development of Ascidians,' Norweg. North Atlantic Exped., 1876–8.

Huntsman, A. G. (1907).—" On the Origin of the Ascidian Mouth ", ' Proc. Roy. Soc.', lxxxvi.

Julin, Ch. (1881).—" Recherches, &c., sur l'hypophyse et quelques organes qui s'y rattachent, I, II ", ' Arch. de Biol.', ii.

—— (1904).—" Recherches, &c., Archiascidia neapolitana, nov. gen. nov. sp.", ' Mitth. Zool. Stat. Neapel ', xvi.

Kingsley, J. S. (1882).—" Some Points in the Development of Molgula manhattensis ", ' Proc. Boston Soc. Nat. Hist.', xxi.

Korschelt u. Heider (1893).—' Lehrbuch d. vergl. Entwickl.', iii.

Metcalf, M. M. (1900).—" Notes on the Morph. of the Tunicata ", Spengel's ' Zool. Jahrb., Anat. u. Ont.', xiii.

Neumann, G. (1913).—" Doliolum ", ' Wiss. Ergebn. d. Deutschen Tiefsee-Exped.', xii.

Pizon, A. (1892).—" Hist. de la Blastogenèse chez les Botryllides (Thèse) ", ' Ann. Sci. Nat. (vii) Zool.', xiv.

Salensky, W. (1893).—" Morph. Studien an Tunicaten, I, II ", ' Morph. Jahrb.', xx.

Sedgwick, A. (1909).—' Student's Text-book of Zoology,' iii.

Seeliger, O. (1893–1907).—" Tunicata ", Bronn's ' Klassen und Ordnungen des Thier-Reichs.', iii, Suppl.

Uljanin, B. (1884).—" Doliolum ", ' Fauna u. Flora, Neapel.'

Van Beneden et Julin (1887).—" Recherches sur la Morph. des Tuniciers ", ' Arch. de Biol.', vi.

Willey, A. (1893).—" Development of the Neuro-hypophysial System in Ciona and Clavelina, with an account of the origin of the Sense-organs in Ascidia mentula ", ' Quart. Journ. Micr. Sci.', xxxv.

EXPLANATION OF PLATES 1–3.

All the figures are of Botrylloides rubrum except fig. 14, *a–d*, on Pl. 2, which are of Clavelina lepadiformis.

In most of the drawings the test and follicles are omitted.

Figures drawn from the same series of sections receive the same distinctive number, followed by a distinguishing letter in brackets, e. g. fig. 4 (*a*), fig. 4 (*b*), instead of fig. 4 and fig. 5.

The position of the section in the total series is indicated by a fractional symbol, of which the denominator represents the total number of sections in the series, and the numerator represents the serial number of the particular section.

The stage of development is indicated by a Roman numeral following the fraction.

Thus fig. 8 (*c*) is given a symbol $\frac{14}{29}$ III, which means that the figure represents the fourteenth of a series of twenty-nine sections, into which an embryo of Stage III was sliced.

LIST OF ABBREVIATIONS

ap, adhesive papillae; *ag*, adult ganglion; *atep*, atrial epithelium; *bs*, blood-space; *cc*, cerebral cavity; *cf*, ciliated funnel; *cl*, cloaca; *dg*, dorsal groove; *ect*, ectoderm; *end*, endoderm; *endd*, endodermal diverticulum; *endos*, endostyle; *extf*, external follicle; *e*, eye; *gs*, gill-slits; *hd*, hypophysial duct; *intf*, internal follicle; *h*, heart; *lpb*, left peribranchial cavity; *m*, mouth; *mc*, muscle cells; *mp*, marginal papillae; *mesc*, mesoderm cells; *nt*, neural tube; *nch*, notochord; *np*, neuropore; *ov*, ovum; *ovd*, oviduct; *ot*, otolith; *oes*, oesophagus; *pl*, precerebral lobe; *ph*, pharynx; *php*, pharyngeal pocket; *phg*, phagocytes; *pt*, placental tube; *rpb*, right peribranchial cavity; *snch*, subchordal cells; *st*, stomodaeum; *sv*, sensory vesicle; *t*, testis; *tc*, test cells; *vd*, vas deferens; *viscg*, visceral ganglion.

PLATE 1.

Fig. 1.—Transverse section of zooid showing female genital organs, including an ovum not quite mature.

Fig. 2.—Transverse section of zooid showing male genital organs, together with a mature ovum and its fertilization duct (*ovd*).

Fig. 3.—Transverse section of embryo (Stage I) showing tubular connexion between outer and inner follicles (' placental tube ') opening into maternal blood-space.

Fig. 4.—Two sections from an oblique longitudinal series (Stage I). $\frac{15 \text{ and } 17}{36.}$.

(*a*) The fifteenth section showing neuropore.

(*b*) The seventeenth section showing anterior dilatation of neural cavity (cerebral vesicle).

Fig. 5.—Section from an oblique longitudinal series (Stage II) showing otolith cell projecting from dorsal wall of cerebral vesicle.

Fig. 6.—Longitudinal section (Stage I) showing subchordal cells.

Fig. 7.—Transverse section (Stage I) showing subchordal cells.

Fig. 8.—Four sections from a transverse series (Stage III) to show origin of atrium. $\frac{9.\,12.\,14.\,16}{29}$.

(*a*) The ninth section through dorsal groove and visceral ganglion.

(*b*) The twelfth section through atrio-cloacal involution and subcloacal cord.

(*c*) The fourteenth section through the peribranchial diverticula of the same, with nerve-cord between them.

(*d*) The sixteenth section through dorsal groove and nerve-cord.

Fig. 9.—Six sections from a transverse series (Stage IV) to show the differentiated parts of the neural complex and their relations to stomodaeum and pharynx. $\frac{44\text{–}6.\,50.\,52.\,55}{84}$.

(*a*) The forty-fourth section. Oral diverticulum of pharynx meeting stomodaeal ingrowth from dorsal groove.

(*b*) The forty-fifth ditto. Extremity of precerebral lobe fused with pharynx behind oral diverticulum. Blind end of hypophysial duct above.

(*c*) The forty-sixth ditto. Front wall of sensory vesicle on right. Precerebral lobe below, hypophysial tube above.

(*d*) The fiftieth ditto. The ventral otolith in sensory vesicle. Ventral lobe and dorsal duct still distinct.

(*e*) The fifty-second ditto. Precerebral lobe merging into cerebral wall. Beginning of visceral ganglion.

(*f*) The fifty-fifth ditto. Hypophysial duct opening into neural cavity above the visceral ganglion (behind and to left of sensory vesicle).

Plate 2.

Fig. 10.—Eleven sections from a transverse series (Stage II) from behind forwards, showing origin of hypophysial duct as a solid rod of cells from front end of cerebral vesicle forwards along roof of precerebral lobe. Upper row, $\frac{23.\,24.\,28.\,32.\,34.\,35}{44}$. Lower row, $\frac{36\text{–}40}{44}$.

(*a*) The twenty-third section. Neural tube behind cerebral dilatation dorsal groove above, pharynx below.

(*b*) The twenty-fourth section. Hinder region of cerebral dilatation, bulging to right.

(*c*) The twenty-eighth section. Middle of cerebral vesicle; visceral ganglion below.

(*d*) The thirty-second section. Front of cerebral vesicle. Hinder end of hypophysial rod above and precerebral lobe below.

(*e, f*) The thirty-fourth and thirty-fifth sections. Hypophysial rod above precerebral lobe, both solid.

(*g*) The thirty-sixth section. Ditto, ditto, but front of precerebral lobe wedged in pharyngeal wall. Dorsal groove losing sharp outline.

(*h*) The thirty-seventh section. Front end of both structures. Notice absence of nuclei in precerebral lobe, marking outer ends of cells. Dorsal groove produced below because of increasing obliquity to plane of sections.

(*j*) The thirty-eighth section. Inner ends of cells of dorsal groove beginning to be cut tangentially (apex of stomodaeum).

(*k*) The thirty-ninth section. Ditto, ditto. Groove extending downwards.

(*l*) The fortieth section. Anterior end of dorsal groove cut horizontally, owing to spherical shape of embryo. The lower end of groove marks the anterior morphological extremity.

Fig. 11.—Four sections from an oblique series (Stage III) from behind forwards to show development of lumen in hypophysial duct and incomplete separation from precerebral lobe. The right side of the sections is in advance of the left side. $\frac{34.\ 39.\ 41.\ 45}{79}$.

(*a*) Sensory vesicle in plane of otolith, with visceral ganglion below and hypophysial tube above, the latter distinctly separated.

(*b*) In front of sensory vesicle. The visceral ganglion behind (here on left) merging into precerebral lobe in front (here on right).

(*c*) Hypophysial duct incompletely separated from precerebral lobe, but sharply distinguishable from dorsal groove.

(*d*) In front of both structures. Oral diverticulum of pharynx meeting stomodaeal invagination of dorsal groove; cf. fig. 9, *a*, *b*, for slightly later stage.

Fig. 12.—Longitudinal section of tadpole (Stage VII) showing hypophysial duct and adult ganglion.

Fig. 13.—Longitudinal section (Stage VI) showing complete disappearance of precerebral lobe in front of larval ganglion, the presence of phagocytes, and the beginning of adult ganglion.

Fig. 14. Four sections from a transverse series through an embryo of Clavelina lepadiformis, from before backwards, to show the hypophysial duct opening into the endodermal pharynx before perforation of the stomodaeum. $\frac{3.\,5.\,10.\,12}{42}$. (Lent by Professor Goodrich.)

(*a*) Stomodaeum above pharynx, cut obliquely. Cf. fig. 9, *a*.

(*b*) Hypophysial tube opening into pharynx. Posterior part of stomodaeal base above. Cf. fig. 9, *c*, in which the precerebral lobe replaces the hypophysial duct.

(*c*) Hypophysial tube to left, sensory vesicle to right, in plane of otolith. Cf. fig. 9, *d*.

(*d*) Hypophysial tube opening on left into cerebral vesicle in plane of eye. At this stage the cerebral vesicle is divided anteriorly, but still undivided behind. Cf. fig. 9, *e* and *f*, in which the cerebral cavity is small, and there is no eye.

PLATE 3.

Fig. 15. Photographs, untouched, of a transverse series of sections from before backwards (Stage IV). The bracketed pairs of sections (*c*) and (*d*), (*e*) and (*f*), are consecutive. All others are alternate (i. e. one section omitted) except the last two (*k*, *l*), between which three sections have been omitted.

(*a*) Stomodaeum above meeting oral diverticulum of pharynx below. Section of tail on apparent left.

(*b*) In series from above downwards: dorsal groove with a fragment of stomodaeal wall, hypophysial duct, a blood corpuscle, precerebral lobe containing lumen, pharynx.

(*c*) As (*b*) but showing front surface of sensory dilatation of cerebral vesicle (pale) on right (apparent left), between hypophysial tube above and precerebral lobe below.

(*d*) Dorsal groove virtually closed; hypophysial tube; front of cerebral vesicle with lumen on apparent left (really right); visceral ganglion on apparent right; hinder part of precerebral lobe, with trace of lumen, above depressed roof of pharynx.

(*e*) Dorsal groove (closed); hypophysial duct closely applied to cerebral vesicle, containing ventral otolith; visceral ganglion; pharynx.

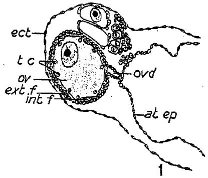
(*f*) Dorsal groove (closed); hypophysial duct opening into cerebral vesicle; visceral ganglion.

(*g*) Hinder end of dorsal groove (closed) sinking farther beneath ectoderm; neural canal; visceral ganglion.

(*h*) End of dorsal groove (= ultimate cloacal aperture), continuous below with front wall of bilobed atrial vesicle (dark); visceral nerve-cord above depressed roof of pharynx (the latter cut obliquely).

(*j*, *k*) Bilobed lumen of atrio-cloacal vesicle above nerve-cord, the lobe to right (really left) extended towards pharynx, the lobe to left (really right) short.

(*l*) Posterior wall of atrio-cloacal vesicle above nerve-cord.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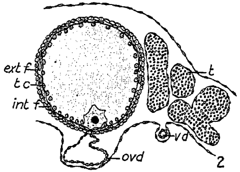
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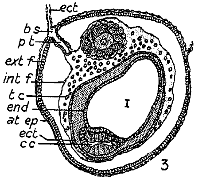
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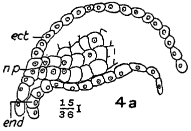
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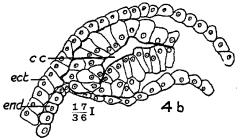
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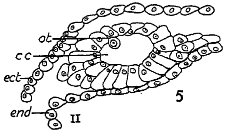
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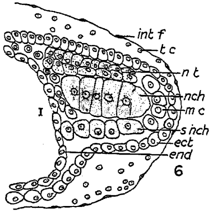
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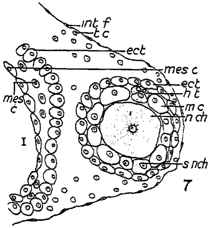
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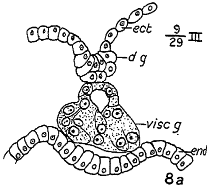
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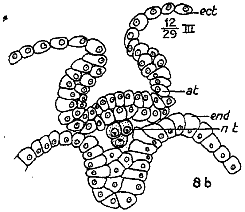
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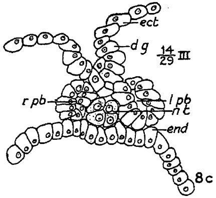
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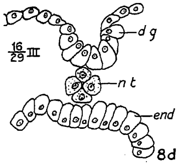
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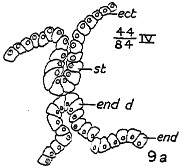
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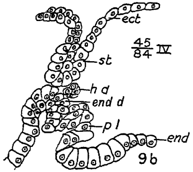
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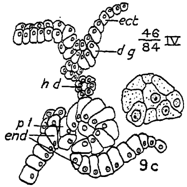
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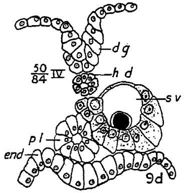
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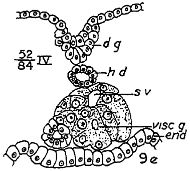
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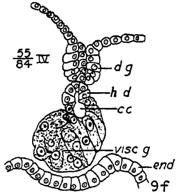
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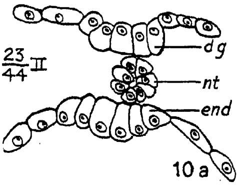

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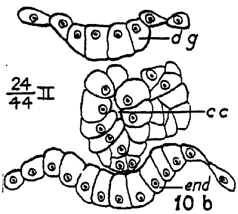

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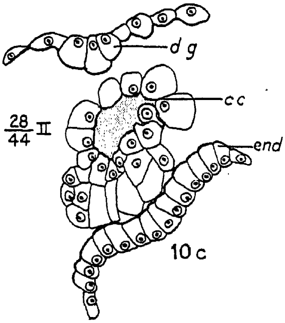

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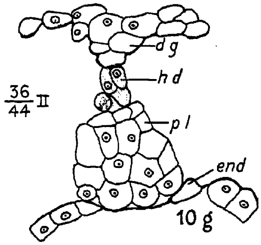

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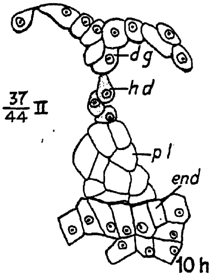

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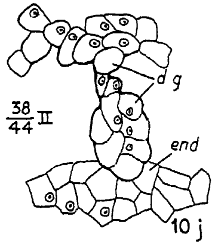

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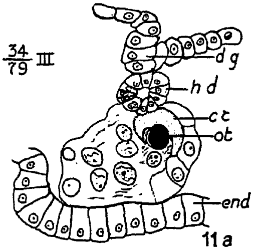

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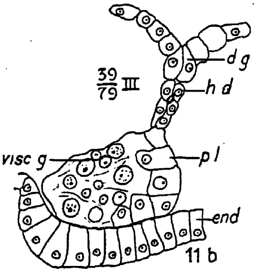

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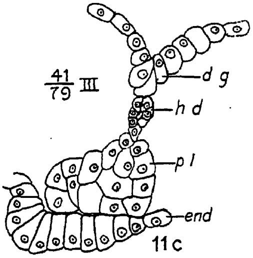

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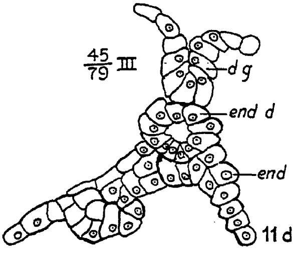

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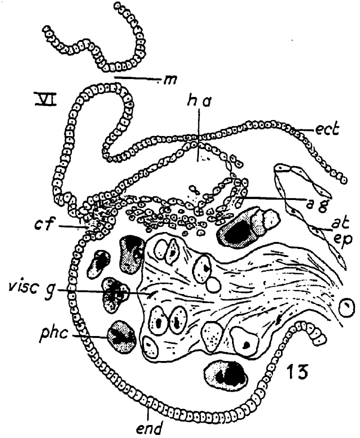

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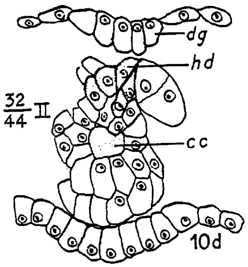
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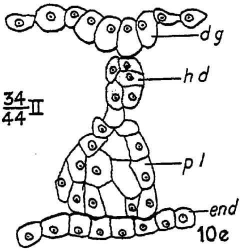
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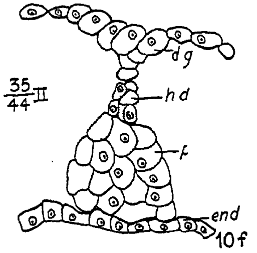
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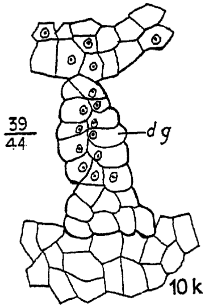
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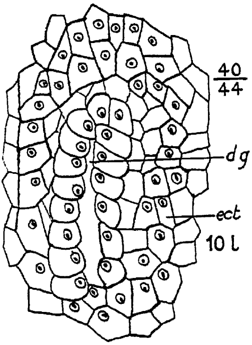
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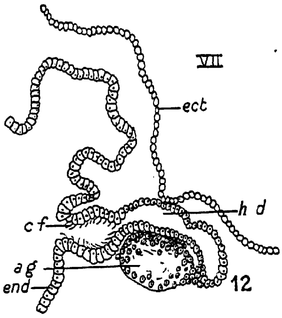
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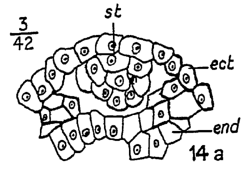
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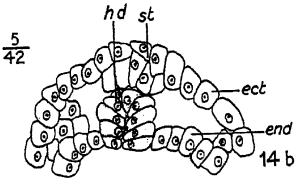
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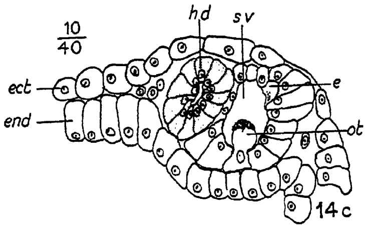
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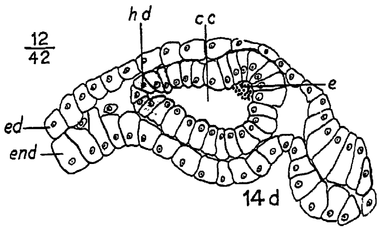
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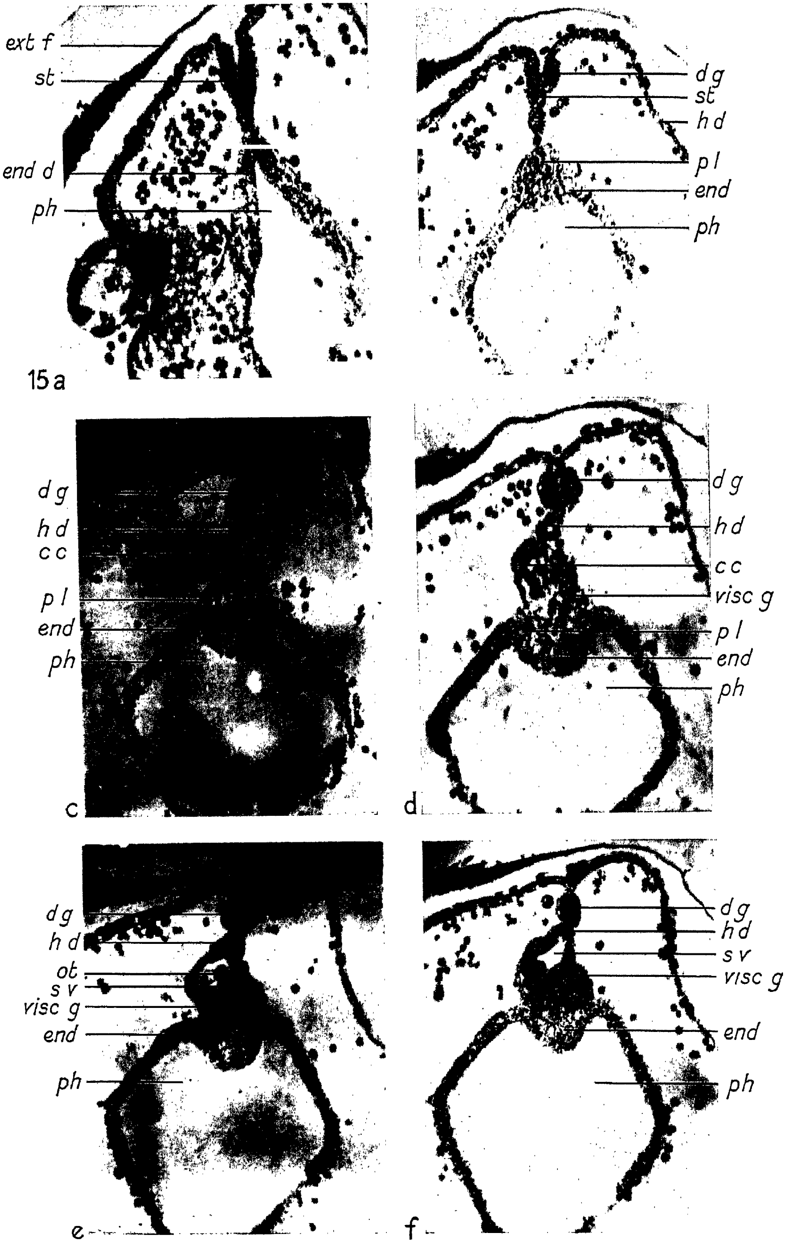
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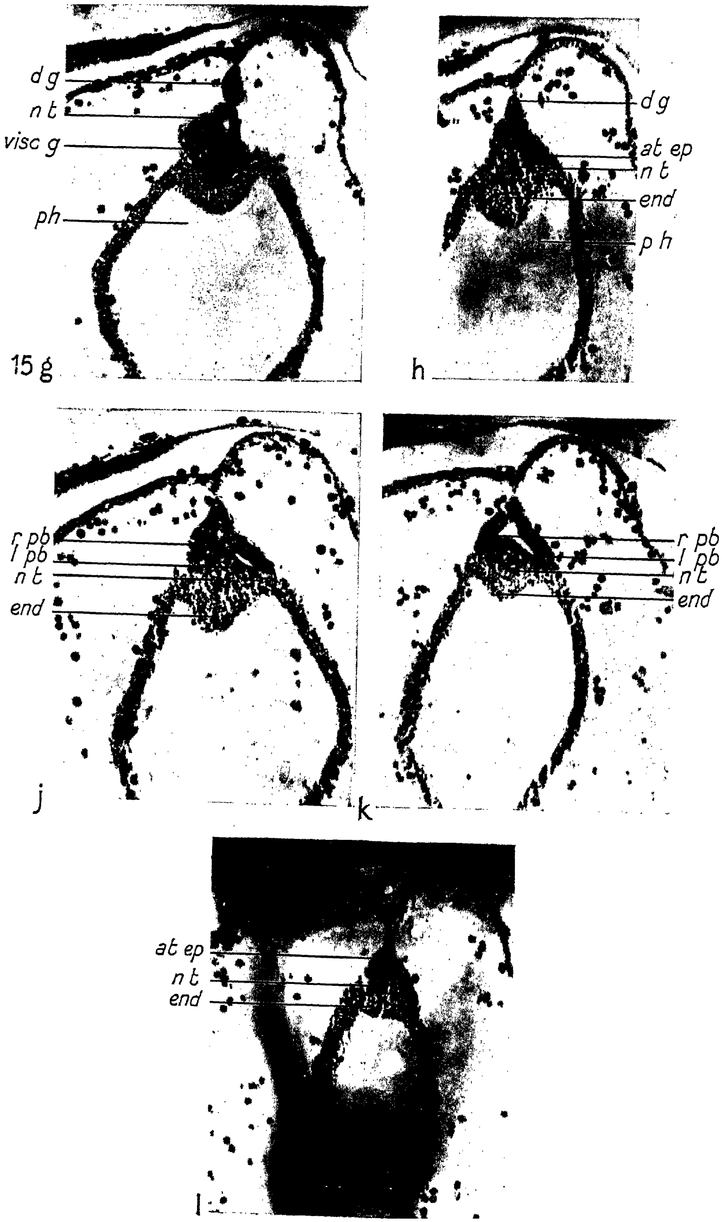
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The Morphology of the Tunicata, and its bearings on the Phylogeny of the Chordata.

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With 13 Text-figures.

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SUMMARY OF ARGUMENT.

As many special points have required detailed examination in the course of this essay, it may be convenient to the reader to be provided with a summary of the main argument.

§ 1. The ancestry of Chordata must be consistent with the systematic sequence: Echinoderm—Hemichordate—Protochordate—Vertebrate, which, at bottom, implies an evolutional progress of plankton-feeding organisms from a fixed condition with external ciliated tentacles and food-grooves to an eventually free and motile state with endopharyngeal apparatus of gill-slits and endostyle.

This implication is corroborated by the secondary character of the coelomic (locomotive) metamerism of Amphioxus, by

the association of an external lophophore with the simplest known condition of gill-slits (Cephalodiscus), and, negatively, by the absence of any proof that pelagic larvae necessitate pelagic ancestors.

The alternative view that Protochordates and Hemichordates are degenerate Vertebrates is precluded by the existence in Vertebrate embryos of vestiges of the complete Protochordate organization of endostyle (=thyroid), epipharyngeal groove (=subnotochordal rod), and tongue-bars (=thymus).

§ 2. The metamorphosis of Ascidians conflicts at various points with the idea that Tunicata have been derived from Amphioxus-like ancestors, especially in the absence of metamerism, the development of lateral atria before the gill-slits, and the independence of larval and adult nervous systems. The neuromuscular relations in Ascidian larvae and Appendicularians are much more consistent with a theory of incipient than of vestigial metamerism. The development of atria before the gill-slits is in accordance with the phyletic history of the Protochordate type of gill-slit, while both the form of the atrium and its mode of development in Amphioxus are probably secondary modifications. The discontinuity between larval and adult nervous systems is unintelligible on the Amphioxus theory, and points to an early divergence between larva and adult in pre-Chordate ancestors, i. e. to a derivation of Tunicates from ancestors with a metamorphic life-history, before the typical Chordate nerve-tube had come into existence.

On the other hand, the form of the gill-slits in Ascidians has undoubtedly undergone great elaboration from the original type, which, as in Amphioxus, was U-shaped, tongue-barred, and synapticulate. In the retention of this type Amphioxus is more primitive than any Tunicate, and in showing the origin of this type from a simple pharyngeal pouch, Balanoglossus is more primitive than either. As the common ancestor could not have possessed more than two to three pairs (the limit in Ascidians), the multiplication of gill-slits in Balanoglossus and in Amphioxus must be regarded as independently acquired since the separation of these forms from the common stock, presumably in correlation with a secondary elongation of the body.

These conclusions with regard to the original form and ancestral number of gill-slits, together with the points previously mentioned with regard to metamerism, atria, and nervous system, become coherent and intelligible on a theory of the derivation of the Chordata from a line of fixed metamorphic ancestors leading from Pterobranchia to Tunicata, Balanoglossus and Amphioxus being derivable from this sequence at different evolutional levels by loss of fixation, retention of the larval symmetry, and independent adaptation to a burrowing life.

§ 3. The theory just outlined imposes the necessity of explaining the Ascidian tadpole as an interpolation in the life-history. The author's Auricularia theory of 1894 is therefore drawn upon to suggest a way in which the muscular Chordate larva may have been evolved from an original ciliated larva of the Dipleurula type, by the substitution of muscular for ciliary means of locomotion. A parallel to this is furnished by certain starfish larvae in which muscular flappings of one or more body-processes have replaced the original ciliary locomotion. A primitive Chordate tadpole may well have arisen from an elongated Auricularia-like larva which acquired the power of undulating from side to side. The organization of such a larva would provide the rudiments of characteristic Chordate features, the circumoral band and its underlying nervous system being regarded as predecessors of the medullary folds, and the adoral band with its ventral loop as the predecessor of the peripharyngeal band and endostyle. By postponement of fixation, the rudiments of the gill-slits, which were primarily organs of the adult stage, may have come into working relations with the predecessor of the endostyle, leading to a synthesis of the full Chordate combination of organs. The loss of the larval tail and nervous system at the metamorphosis of an Ascidian would thus correspond to the loss of muscular appendages and larval nervous system at the metamorphosis of a starfish larva. On the other hand, the persistence of these structures in other

Chordata would be attributable to the loss of fixation and metamorphosis and consequent survival of the larval type of organization (paedomorphosis).

This chapter closes with a reinterpretation of the development of the endostyle in Amphioxus, which is confirmatory of its origin from the ventral loop of the Echinoderm adoral band.

§ 4. The prevalent view of the primitive nature of Appendicularians is here examined in detail. The horizontal tail is a common feature of Synascidian larvae, but the dextral twist of the intestinal loop, in opposition to the sinistral twist in fixed Ascidians, militates against an origin of Appendicularians directly from that source. On the other hand, the passage of the nerve-cord across the right side of the oesophagus is found again in the larva of Doliolum, together with a straightening out of the intestinal loop and even a dextral twist in many species. This indication of affinity is corroborated by agreement between the two types in what appears at first sight to be a primitive feature, the position of the endostyle in front of the gill-slits; but this, in Doliolids, is shown to be a secondary modification, caused by the backward rotation of the cloaca and the withdrawal of the peribranchial cavities from the front part of the pharynx. From the mode of working of the pharyngeal apparatus, and from the structure of the endostyle, it is shown that the Appendicularian pharynx has been derived from that of Doliolidae, which is itself derivable from that of normal Ascidians. The oozooid of Doliolum possesses the equivalent of only two pairs of gill-slits, so that the single pair of Appendicularians represents no great reduction. The sequence is not reversible.

In addition to these points the differentiation of the Appendicularian ectoderm into oikoplastic and non-oikoplastic areas is a unique and extremely modified character. As the non-oikoplastic area lies posteriorly, and receives the apertures of gill-slits, intestine, and gonads, it is claimed as the equivalent of a shallow atrio-cloacal cavity, like that of Doliolum, from which it has probably been derived by suppression of the cavity and eversion of the epithelial lining (cf. gastrozooids of Doliolum). The dorsal hood of various Appendicularian genera is regarded as a modification of the dorsal outgrowth of the cloacal rim which carries the creeping stolon in Anchinia and the free buds in Doliolum.

On this theory, the 'Haus' of Appendicularians, formed from the oikoplastic area, becomes the exact equivalent of a complete Doliolid test, which has been shown by Uljanin to be subject to exuviation and renewal like the Appendicularian 'Haus'. The primitive form of house is shown to correspond with the requirements of this theory by possessing two opposite apertures, one oral and the other posterior.

The mixture of larval and adult characters which is so characteristic of the young Doliolum, and is a result of the abandonment of fixation, provides the key to Appendicularian structure, which combines the simplified body of an adult Doliolum with retention and specialization of the larval tail. The alleged metamerism of the Appendicularian muscle-cells is examined, and Martini's theory of 'Eutely', as a substitute for 'partial Neoteny', is criticized.

§ 5. This chapter deals primarily with a number of anomalous and puzzling endodermal glands, derived from the pharynx immediately behind the endostyle, and either retaining that connexion or acquiring a secondary communication with the exterior by fusion with the ectoderm. These are the 'pharyngeal pockets' of Fritillaria, the 'oral glands' of Oikopleura, and the 'club-shaped gland' of Amphioxus. All are claimed to be vestiges of the Ascidian organ of budding known as the 'epicardium', with a reservation in the case of the Oikopleura glands, owing to uncertainties with regard to their development.

In the case of the Polyclinid Ascidian Euherdmania, which possesses two separate epicardial tubes, it is known that one undergoes a glandular modification, apparently in consequence of the loss of its original function. In Molgulid Ascidians both epicardial tubes have been arrested in development, and, after fusing with one another in the usual way, have been transformed into a closed renal organ; and the endocrine modifications of endostyle, tongue-bars, gill-pouches, &c., in Vertebrates furnish additional illustrations of the tendency of vestigial organs of the Protochordate pharynx to be transformed into glands of various kinds.

It is suggested that the endodermal 'cement-glands' of larval Teleostomes may be further derivatives from the same source, their original position having apparently been ventral and postoral.

§ 6. The asymmetry of the larval Amphioxus is a remarkable phenomenon which affects the interpretation of many transitory features as well as the general relationships of Amphioxus. It has therefore been restudied and is here explained as the consequence of a secondary reduction of yolk in the egg, entailing premature hatching and the improvisation of a larval feeding mechanism. A great enlargement of the mouth, and special ciliation of its entrance, seem to form the basis of this mechanism, which, under the circumstances of the case, involves a temporary dislocation of all the adjacent parts, and is held to have entailed changes which have left a mark on the permanent organization of the adult. The club-shaped gland probably functions as a source of mucilage in place of the imperfectly developed endostyle. The precocious multiplication and distension of gill-slits, as part of the feeding mechanism, are held to be responsible for the peculiar form and mode of development of the belated atrium. These considerations, added to the presence of a vestigial epicardium, lead to the conclusion that the ancestors of Amphioxus were essentially primitive Ascidians, and that Amphioxus itself, while retaining some primitive characteristics, is another example of paedomorphosis. The 'caudal appendage' or 'urostyle' of Asymmetron, which carries the larval fin, is claimed to be the homologue of the Ascidian larval tail, of which the larva of Amphioxus retains a vestige in its provisional caudal fin.

§ 7. This chapter surveys the modes of budding in Tunicata, since any evidence that the epicardium was of relatively late origin within the group would be inconsistent with the conclusion that Amphioxus possessed a representative of it. Apart from two cases, in which the evidence is doubtful, it is shown that there is a sharp cleavage of the fixed Ascidians into two groups, here called Endoblastica and Periblastica, in one of which the inner vesicle of the buds is derived from pharyngeal outgrowths (epicardia), and in the other from peribranchial outgrowths, and that these differences correspond with equally clear distinctions between the two groups in regard to adult structure and larval characters. These two groups must be derivable from some third stock more primitive than either, but such a stock among fixed Ascidians is unknown and may possibly be quite extinct.

On the other hand, the mode of budding in Thaliacea combines both types found in Ascidiacea, owing to the fact that the internal organs of the buds are built up from paired rudiments derived both from the pharynx and the cloaca of the parent. There is every reason to believe therefore that the primitive Tunicata, from which both the Thaliacea and Ascidiacea were derived, resembled the Thaliacea in possessing both pharyngeal and peribranchial outgrowths in the stolo prolifer with which they were doubtless provided. Thus a pair of epicardia probably existed in the earliest stock of Tunicata, together with a pair of peribranchial diverticula.

If Amphioxus is correctly regarded as a paedomorphic representative of that primitive stock, its possession of vestigial epicardia is accordingly consistent. Lankester's 'atrio-coelomic funnels' may conceivably represent the corresponding pair of peribranchial diverticula, the dislocation between epicardial and peribranchial outgrowths being explained by the precocious specialization of the former for larval purposes and the retarded development of the atrium.

It is suggested that these two pairs of elements in the early Tunicate stolon may have originated in the fixed ancestors of the Protochordata as regenerative discs or pockets at the base of the pharynx and atrium respectively, to serve for the rejuvenation of the thoracic region after periodic atrophy (cf. Diazona, Distaplia, &c.). When the ectoderm of the primitive stolon became too specialized to play an important part in budding, these regenerative pockets may then have extended into the stolon and taken over its function, collectively at first (Thaliacea), alternatively in the two sections of Ascidiacea, the inner (or epicardial) elements persisting in those which retained the original median stolon (e.g. Clavelinidae), the outer (or peribranchial) in those which widened the original base of fixation and developed their buds laterally (e.g. Botryllidae).

In a subsequent communication the author will deal with the origin of the Chordate nervous system and with the various cephalic organs associated with it as hypophysis, wheel-organ, subneural gland, &c.

§ 1. PHYLOGENY AND BIONOMICS.

The development of Ascidians has hitherto been interpreted as an example of adult degeneration consequent upon the assumption of sessile habits by a free-swimming ancestor. The organization of this ancestor is generally assumed to have been more or less like that of an Amphioxus or an Ammocoete, sharing an open endostyle and ciliated gill-slits with the former, and cerebral sense-organs and absence of an atrium with the latter, together with the neuromuscular metamerism of both. The tailed larva is supposed to 'represent' such an ancestor, which, having fixed itself to a rock, was eventually superseded as an adult by the modern sessile creature. As larvae are usually smaller than adults, appeal is made to reduction of size as a factor capable of accounting to some extent for the manifest imperfection with which the actual larvae record the features of their former sires.

The most striking imperfections of the biogenetic record which have to be explained on this hypothesis are the following: (1) absence of coelomic and neuromuscular metamerism; (2) development of lateral atria before the gill-slits; and (3) independence of larval and adult nervous systems.

These, possibly, are small matters compared with the possession by Tunicate larvae of the typical Vertebrate combination of notochord, gill-slits, and neural canal. At the same time it is well to realize that since Kowalevsky's demonstration of the Vertebrate affinities of the Tunicata sixty years ago our knowledge of various types of organization connecting Vertebrates and Invertebrates has greatly increased, and the idea that the ancestor of Vertebrates was a kind of free-swimming pelagic Annelid has long been discarded as untenable. Below the Protochordata (i. e. Amphioxus and the Tunicata) come the Hemichordata, with gill-slits, but little or no notochord and neural canal; and below Hemichordata come the sedentary Echinoderms with neither notochord, neural canal, nor gill-slits, yet all linked in a sequence of relationships by common features of structure and development which render their consideration essential in a discussion of Tunicate origins. Even if the Enteropneusta were unknown, the anal blastopore, the azygos coelomic water-pore, and the mesodermal skeleton would remain as remarkable bonds between Echinoderms and the Chordata.

If therefore we can disregard for a moment all theoretical prepossessions, we must admit that, on the face of things, systematic morphology points to conclusions very different from the ideas of sixty years ago. Firstly, the myomerism of Amphioxus and the Vertebrates is probably a secondary phenomenon, derivable from the simple, tripartite organization of the Enteropneusta (Morgan, 1894; MacBride, 1898, 1909)—a conclusion which, physiologically expressed, implies a change from relatively feeble to relatively vigorous means of locomotive activity. Secondly, the gill-slit apparatus of Vertebrates is admittedly in its origin an apparatus for filtering plankton as a food-supply, and in Cephalodiscus, as Harmer pointed out in his famous 'Challenger' appendix (1887), we have a Hemichordate that bridges the gap between the earlier, tentaculate, type of plankton-collecting and the later endopharyngeal type with ciliated gill-slits or stigmata. If this bridge be regarded as fairly safe—and without it we can see no way across—the conclusion again implies an evolutional origin of Vertebrates from ancestors of essentially sessile habits, for there is hardly an exception to the overwhelming rule that lophophores are the feeding organs of sedentary or tubicolous types, of which Echinoderms were amongst the earliest examples.

The views advanced by Dohrn and Gaskell that Protochordates and Hemichordates are the result of a retrograde evolution from typical free-swimming Vertebrates are inconsistent with the fact that the Vertebrate pharynx from Cyclostomes upwards gives substantial ontogenetic evidence of descent from a previous Protochordate condition, the extent of which is hardly yet appreciated as fully or as widely as it deserves. That the thyroid gland is the modified remnant of an endostyle is generally admitted, but the equally cogent evidence that the metameric rudiments of the thymus betray an origin from vestigial tongue-bars, as suggested by Willey (1894, p. 29), has been neglected. The developmental evidence in such a form as Spinax (Fritsche, 1910) is unequivocal (cf. Brachet, 1921). Less certain, but not negligible, is the evidence which points to the epipharyngeal groove as the functional predecessor of the subnotochordal rod, the chief difficulty being the greater extent of the latter—a discrepancy which may possibly be explained by the embryonic vestige having become secondarily utilized for the formation of a sub-vertebral ligament and thus extended beyond its original limits.

There is of course nothing novel in regarding the ancestry of Vertebrates as intimately connected with that of Echinoderms and Hemichordata, but hitherto the relations which I have just emphasized between these types of organization and a sedentary, or relatively sedentary, mode of life have been thrust aside by a purely speculative prepossession in favour of ‘ free-swimming pelagic ancestors ’. So in 1894 we had my Auricularia ancestor, in 1897 Masterman's Actinotrocha ancestor, and in 1909 MacBride's, which, though claimed to have been constructed synthetically by ‘ adding the arms (of Cephalodiscus) to the collar of a short-bodied Balanoglossus ’, can be seen from its portrait (MacBride, 1909, p. 336, fig. 10) to be identical with an Actinotrocha, except in lacking those details of structure which alone render possible a pelagic career for that anomalous larva (locomotive telotroch, hood-like pre-oral lobe to protect and guide the food-currents, &c.). Yet in each of these theories we neglected two vital points, firstly that by magnifying tiny larvae into effective adults we exceeded the critical limits possible for simple ciliary methods of locomotion and feeding; and secondly that, had these putative adults ever become mature and laden with gonads, it would have been physically impossible for them to keep afloat, unless the original type of ciliary locomotion were reinforced or replaced by muscular. MacBride's ancestor, indeed, might well have resorted to wriggling, and so initiated that metamerization of its trunk which was required to make a Vertebrate of it; but it is obvious that violent locomotion, after the manner of a pelagic Annelid, would have been utterly inconsistent with the rest of its organization (ciliated arms for catching plankton).

There is a famous sentence in the preface to Thomas Huxley's 'Manual' which the late Professor Weldon was fond of quoting: 'The growing tendency to mix up aetiological speculations with morphological generalizations will, if unchecked, throw Biology into confusion.' I think it might be said with equal truth that the tendency, now happily past, to restrict Zoology to 'morphological generalization' has itself caused a good deal of ill-founded evolutional speculation.

That the morphological theory of adult recapitulation need no longer limit us in the study of phylogeny, I have already attempted to show (1922); but in view of Professor MacBride's reply (1926) I may perhaps add the following remarks.

In his text-book of Embryology and elsewhere (to which detailed references are given in my paper on the subject) Professor MacBride had selected a number of cases which seemed to him to be free from ambiguity as tests of the truth of the theory as he regarded it. In addition to other criticism, I took the whole series of these examples and their ancestors from Professor MacBride without exception or qualification, and showed that, contrary to his theory, the test of special resemblance between the larval stage of the 'descendants' and the adult stage of their 'ancestors' broke down in every case

(1922, § 11). In such a case as that of Portunion the absence of legs on the last thoracic segment is indeed so unequivocally a larval character throughout the Isopoda, and not a normal adult character, that argumentation on the point is impossible.

Yet in an article devoted to this subject and to my criticism Professor MacBride ignores the results of this crucial test altogether, and devotes his 'limited space' (actually some thirteen pages) to repetition of the general arguments to be found in his earlier writings, with additional illustrations, always readable and full of interest, but in no case directed towards the point at issue.

In view of these facts I am entitled to repeat that the theory of adult recapitulation is dead, and need no longer limit and warp us in the study of phylogeny. Instead of assuming that the phases of a given ontogeny represent a compressed succession of adult ancestors, I shall simply assume (1) that under similar conditions egg, larva, and adult of one ontogeny inherit—or tend to preserve—the characters of egg, larva, and adult of a previous ontogeny; (2) that, instead of new characters tending to arise only towards the end of the ontogeny, they may arise at any stage of the ontogenetic sequence; and (3) that, instead of new characters always tending to push their way backwards in the ontogeny, they may extend into adjoining stages in either direction, either backwards from the adult towards the larva and the embryo (tachygenesis) or forwards to the adult from the embryo and the larva (paedomorphosis).

We can now proceed to deal with the problem immediately before us, the current views on which are so compactly and authoritatively expressed in the article to which I have referred: 'It is really impossible for a competent marine biologist, and especially for a specialist in Tunicata . . . to deny the fact that the Tunicate tadpole indicates a former free-swimming ancestor of the Tunicata, which was provided with a notochord' (MacBride, 1926, p. 470).

To deny a fact is, of course, impossible; but I will try to show that certain facts, which are demonstrable and undeniable, admit of, and in my judgement demand, a very different interpretation.

§ 2. The Metamorphosis of Ascidians.

Before comparing the life-history of Ascidians as a whole with that of lower groups, it will be convenient to examine the points already mentioned (p. 52), since an intimate relationship between the Tunicata and Amphioxus cannot be doubted, and the points raised are essentially differences between the modes of development of these two types.

Mere reduction of size from an assumed ancestral condition seems to be quite inadequate as an explanation in this case, since the larvae of Amphioxus are just as small as those of Ascidians, if not smaller, yet in them successive coelomic sacs are clearly defined, the gill-slits, or many of them, are perforated before the atrium develops, and there is no distinction to be drawn between larval and adult nervous systems.

An important condition undoubtedly is the extreme specialization of the Ascidian larva for larval purposes, which in this case are two only, viz. locomotion and fixation. The larva does not feed, its mouth being covered by a coating of test-substance (Seeliger in Bronn, p. 812); locomotion is effected, not by ciliary action, or by serpentine movements of the whole body, but by a tail which is sharply defined from the visceral region; and the ventral sucker, by which fixation is accomplished, is given its terminal position in front by a temporary suppression of pharyngeal development, rotation forwards of the endostylar region, dislocation of the mouth to the dorsal side, and complete obliteration of the pre-oral lobe.

To these structural peculiarities of the Ascidian larva must be added the differentiation of the embryonic neural tube into two successive and independent nervous systems, one subservient to the larval, and the other to the adult organization, a remarkable and almost unique phenomenon. Possibly this profound specialization of the larva is causally connected with the direct and ‘ determinative ’ character of the early development, which contrasts so markedly with that of Amphioxus.

When the egg itself is already differentiated into zones of predetermined fate, it is also useless to look for vestiges of ancestral structures like pre-oral and collar enteroceles, which are wanted functionally neither by larva nor adult.

The absence of regular neuromuscular metamerism in the tail of Ascidian larvae (and in the Appendicularians as well, cf. § 4, end) is, however, a different matter, for metamerism is undoubtedly a means of securing locomotive efficiency, and it is hardly likely that it would be discarded, when once it had been acquired, so long as the larvae at any rate required to swim. It would seem, therefore, that the imperfect segmentation of the Tunicate tail (for details see Seeliger, 1900, and Ihle, 1913) is a case of metamerism in the making, and not of a secondary degeneration, since, as already remarked, the argument based on small size cannot with consistency be invoked in support of the latter theory.

The second difference between the development of Tunicata and Amphioxus concerns the atrium and gill-slits. The atrium of Tunicata is essentially and primarily a pair of lateral canals or chambers. The incorporation of the mid-dorsal integument between them, including the anal aperture, to form a cloaca, is admittedly a secondary phenomenon. The whole peribranchial complex is now known to be of purely ectodermal origin in the embryos of every type of Tunicate.

The earliest—and still, perhaps, the most generally accepted—theory of the larval atrial canals was based on the simple arrangements prevalent in Appendicularians, in which the pair of spiracles, on the analogy of some Vertebrate gill-slits, could be regarded as a pair of similar structures, formed by the fusion of endodermal pouches with ectodermal pockets, the spiracular ring of cilia marking the boundary between the two elements. Although the basis for this interpretation has since been undermined by Delsman's observation (1910, 1912) of the endodermal unity of the whole structure, it remains true that the lateral atria of Ascidians on this theory have been regarded as secondary expansions of the outer ('ectodermal') pockets, and the pharyngeal stigmata as secondary multiplications of the originally simple perforations (Fol, 1872, pp. 5–6; Seeliger, 1893, pp. 391, 398; Julin, 1904, p. 607). On this theory Tunicata are primitive Vertebrates with a single pair of 'true' gill-clefts.

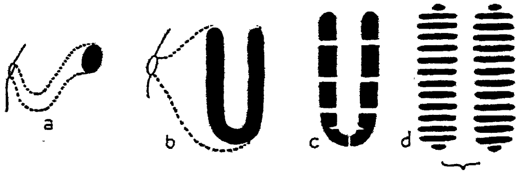
This view was accepted by Willey in his first paper on Ascidian development (1892, p. 517), but abandoned in his larger paper of the following year (1893, i) owing to further reflection on his discovery of the U-shaped form assumed by the primary protostigmata in Ciona, with its striking resemblance to the tongue-bar formation in Amphioxus. He concluded that 'the stigmata in the branchial sac of the Ascidians are derived from and represent three pairs of primary gill-clefts' (l. c., pp. 335, 336, 341). With this conclusion Willey associated an attempt to homologize the atrium of Tunicata with that of Amphioxus, basing his argument on the groove-like rudiments of the larval canals in Clavelina. Two years later, however, he again changed his mind as to the number of primary gill-clefts, which he once more restricted to a single pair (1894, pp. 232–4) and emphasized the tubular character of the atrial 'inpushings' as against an origin from branchial grooves (l. c., pp. 211–12), the case of Clavelina being relegated to a footnote (p. 241). This appears to be Willey's final interpretation, since he reaffirmed it five years later: 'There are strong grounds for the interpretation of the numerous branchial stigmata as having originated by the subdivision of a single pair of gill-slits which persist in their undivided condition in Appendicularia' (1899, p. 238).

I confess I have never been able to appreciate the reasons given by Willey for this reversion to his earlier view, for the differences which he showed to exist between Molgula and Ciona, as regards the order of development of the protostigmata, seem to be much less significant than the agreement between these Ascidians in showing three pairs of primary U-shaped gill-slits. This phenomenon has since been claimed to occur in all types of 'simple' Ascidians (see Julin, 1904), and a renewed survey of the facts only strengthens the illuminating comparison which Willey originally drew between this mode of origin of the Ascidian rows of stigmata and the development of the tongue-barred gill-slit of Amphioxus (and Balanoglossus) with its potentialities of subdivision by synapticula (see below, p. 68). Willey's later restriction of the number of similar slits in Ascidians to one 'primitive' pair was based on minor differences of behaviour and sequence in Molgula and Ciona; but his selection of the first pair in Amphioxus as alone represented in Ascidians is invalidated at once by the fact that the first pair in Amphioxus (one member of which is claimed to be the club-shaped gland) arises and atrophies without ever undergoing the tongue-barred modification displayed by the rest of the series (Willey, 1894, p. 148).

It is true that Julin's scheme of Ascidian evolution ignores this Amphioxus-trait altogether, and in its broad outlines, which are consistently based on other data, agrees essentially with Willey's final interpretation, so that the two views may be thought to be mutually corroborative. Julin's scheme, however, is purely formal and deductive, assuming as self-evident propositions that a condition with one pair of gill-clefts is more primitive than a condition with two pairs, and a condition with two pairs more primitive than one with three pairs. So he gives us in the first line the Appendicularian condition, with a single pair of tubular spiracles, and the other pelagic Tunicates as very debatable derivatives from it; in the second the Archiascidia-Clavelina-Distaplia series, with stigmata derived from two pairs of primary slits; and lastly the Ciona-Molgula-Styela series with three pairs of primary slits, in which the U-shaped form attains its most complete expression. An orderly arrangement of numerical facts is always arresting, but when it conflicts with the order revealed by more substantial facts of structure, it is not doubtful which of the two must give way: priority in Tunicate classification must be given to those characters which most intimately link the group to its primitive relatives. Willey's discovery of the tongue-barred, synapticulate gill-slit of Amphioxus in the pharynx of certain simple Ascidians has given us such a character, not in its primitive simplicity, but disguised by a number of developmental and evolutional peculiarities which demonstrate its transmission to the group from without, rather than its origination from within. If this be admitted, then the Appendicularian, with its mathematical simplicity, is morphologically degraded, and the ancestral Tunicate was polytrematic, like Balanoglossus and Amphioxus, its pharynx possessing a series of at least two, possibly three, pairs of tongue-barred, synapticulate gill-slits.

It is curious that Damas, in his concluding paper on the Ascidian pharynx (published, like Julin's, in 1904), should have come very nearly to the position here maintained, and then, like Willey, have dropped back into the current Appendicularian theory, with the formula 'Les Tuniciers ne possèdent qu'une seule paire de fentes branchiales' (1904, pp. 820, 825). For he first confirmed and extended my early observations (1892) on the apparently independent origin of the protostigmata in Styelopsis (=Thylacium) and Botryllus, and their homology with the indefinite number of perforations in Pyrosoma; and then, after dealing with the various modifications of the type of development discovered by Willey in Ciona, remarked upon the close similarity between the series of three independent crescentic protostigmata of Molgula and the tongue-barred slits of Amphioxus (p. 822). He even admitted that 'logiquement nous devrons admettre que le type le plus primitif est représenté par la Molgule, dont le développement de la branchie présente, parmi les protostigmates, la régularité la plus grande'.

TEXT-FIG. 1.



Showing the evolution of the tongue-barred, synapticulate, gill-slit.
a, Cephalodiscus; *b*, Balanoglossus; *c*, Amphioxus; *d*, Ascidian.
In *a* and *b* the walls of the gill-pouch are indicated by dotted lines.

Damas gave two principal reasons for not adopting the one conclusion which he admitted to be logical: (1) the difficulty of accepting the development of Molgula as more primitive than that of Ciona, when its adult organization is more anomalous; and (2) the occasional connexion between protostigmata I and IV in Ciona before they give origin to II and III.

The first of these reasons is clearly invalid. The adult organization of all modern Ascidians known to us is modified in one way or another (not even excepting Julin's remarkable Archiascidia), and there are several features of Molgulid structure, notably its tendency towards bilaterality of the gonads, in which it is quite conceivably more 'primitive' than Ciona. Moreover, the argument is at variance with established facts: it would prevent us, for example, from accepting Auricularia as a more primitive type of larva than Bipinnaria (although the latter goes through an Auricularia stage), since the adult Holothurian structure is admittedly more modified than that of Asteroids.

The second reason confessedly had great influence with Willey himself (1893, p. 322), as it has had with two subsequent workers—Damas (1904, p. 824) and Huntsman (1913, p. 441). It demands therefore a closer examination, although the connexion is known and admitted to be a mere abnormality, and should no more influence morphological interpretation than any other casual abnormality. This view, though with reservation, has indeed already been foreshadowed by De Selys Longchamps, who has seen but one example of the abnormality in Ciona and two in Ascidiella (1900, p. 678). Taking the normal development of Ciona and Molgula as our basis, we see the pharynx perforated by a succession of imperfectly U-shaped slits, which recall the tongue-barred synapticulate slits of Amphioxus, and ultimately attain complete resemblance to them. Let us assume that in their completed form the two structures are fully homologous. Then we must recognize that the development of the slit in Ascidians has been modified in a quite specific manner. Each gill-slit is represented by a genetically connected pair of protostigmata which are characteristically curved and orientated towards one another like the two halves of a tongue-barred slit, which has already been divided by fusion of the tip of the tongue-bar with the ventral wall of the slit. If the two halves appeared simultaneously, it would be a simple case of precocious or direct development (cf. Doliolum, p. 104), but normally this is not the case. Probably by adaptation to the conditions of interstitial growth in the narrow atrium, the gill-slit develops on the instalment system, first one-half (the parent protostigma), and then the second half (the daughter protostigma), by curvature and upgrowth from the ventral end of the first. To avoid further confusion, let us name the first or parental protostigma a 'hemitreme',[1] and note that each hemitreme possesses a special growing point at its ventral end, by which its growth into a complete tongue-barred slit is completed. In Molgula the hemitremes arise successively, like the gill-slits of Amphioxus, or rather of Balanoglossus, but in Ciona and its relatives the first two hemitremes arise simultaneously. In Molgula also and its allies the successive hemitremes are all orientated alike, with their growing points behind, while in Ciona and its allies (except Perophora) a mutual symmetry is exhibited by the first two hemitremes (in obvious relation to their simultaneous development), so that their growing points are directed towards one another. Occasionally these curving, growing ends accidentally meet and fuse (is it surprising ?), and produce the illusion of a large tongue-barred slit, which has sent Willey and his followers so far astray in their interpretations (cf. Huntsman, l. c., p. 441 : 'Willey concluded from this that the first four protostigmata were derived from a single primary gill-slit. This view seems to be justified by the facts'). But that this putative slit is an illusion, and not a morphological unit, is clearly shown by the normal independence of its two halves (cf. Julin, 1904, figs. 23–5), by the irregular and variable forms which the temporary fusion takes (Willey, 1893, figs. 7, 8, 16, 20), and by the absence of any observation that it ever arises from a single perforation or rudiment. Moreover, in Perophora (Damas, 1904) the two primary perforations become hemitremes of a slightly modified type, symmetrically orientated back to back, so that their principal growing points curve outwards instead of inwards, exactly the reverse of their direction in Ciona!

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[1] This word was first used by Lahille (1890, p. 26) and defined as 'un tréma en voie de formation'. No confusion can arise from the fact that Lahille erroneously applied it to the ciliated pits along the 'gill' of Salpa.

It follows that the number of gill-slits represented in the Tunicate pharynx should be determined without regard to this abnormality in Ciona, and with reference solely to the number of actual hemitremes observable, whenever the development of the pharynx takes place through the intermediation of these structures. This number in Ciona and Molgula alike is three pairs (two in Perophora).

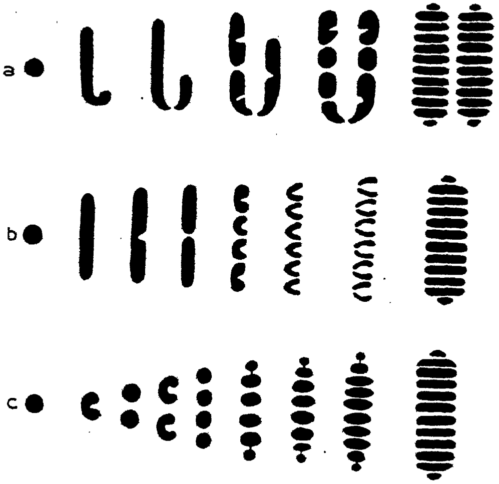
If now we turn to Huntsman's ingenious diagram (1913, fig. 2) where all the known types of development of stigmata are synoptically assembled and classified, we see that in the series of 'Ptychobranchia' (=Stolidobranchia of Lahille) a gradual evolutional change has taken place in the mode of development, the hemitremes (represented by crooks) of the Molgulids (=' Caesiridae') being replaced by couples of long and short protostigmata in Cynthiids (=' Tethyidae'), in which the genetic connexion between the two is broken at a very early stage, and in the hinder part of the pharynx discontinued altogether. Finally, in Styelopsis (Dendrodoa) and the Botryllidae the protostigmata are represented as independent from the beginning.

The completion of this series, largely by Huntsman's own observations on Boltenia and Styela, makes it clear that the metameric regularity of the protostigmatic units in Styelopsis and Botryllus (as well as Pyrosoma), to which I drew attention in 1892, is a secondary phenomenon. Damas (l. c., p. 820), in his comments on my work, inadvertently says that I considered 'chacun des stigmates du Pyrosoma comme une fente branchiale', a remark which, if true, would imply that I also held the same view of the protostigmata of

Ascidians generally. Reference, however, to the introductory paragraph of my paper (l. c., p. 505) will show that this was never my opinion. On the contrary, what I did say, when introducing the new term 'protostigmata', was simply: 'It cannot be denied that these structures present striking analogies with the true gill-clefts of Amphioxus and the lower Vertebrata' (l. c., p. 510). At the time in question zoologists were working under Van Beneden and Julin's conclusion that true gill-clefts in Ascidians had disappeared. It is not irrelevant to remark that Willey's paper on Ciona and mine on Styelopsis and Botryllus were read, as it happened, at the same meeting in 1892, and neither of us had any previous knowledge of the other's work. From the moment of hearing Willey's results, I have never ceased to regard his discovery of the crescentic type of slit as furnishing the real key to Ascidian morphology, and my own as being in some way merely a special modification of it. If I have now succeeded in putting Willey's discovery on its true basis, the controversy as to the number of gill-slits in Ascidians must automatically cease. For whether Damas and Huntsman are right in supporting, or Julin is right in qualifying, my account of the apparent independence of the protostigmata in Styelopsis and Botryllus, it is now perfectly clear that these structures represent, not gill-slits, but gill-slits bisected. The primitive unit in Tunicate ancestors was a tongue-barred slit, as in Amphioxus, which formed its stigmata by synapticulae. It then became a hemitreme, which, curving dorsally, divided into two protostigmata before it had assumed its final form, and each protostigma produced a row of stigmata by subdivision. Then the protostigmata became independent developmental units, each producing stigmata at first by septation, and in later stages of growth by a kind of budding process both dorsally and ventrally. Finally, the climax of this transformation is reached in Clavelina and its allies, in which even the half gill-slit (or protostigma) fails to complete itself, the unit of formation becomes a self-reproducing stigma, and the whole process of development is so modified that interpretation in terms of gill-slits is largely arbitrary (see Julin, l. c., figs. 9–20).

The two first pairs of larval perforations in Clavelina are generally admitted to be homologous with the two initial pairs in Ciona; but whereas Ciona develops a third pair, and the three pairs divide into six pairs of protostigmata, which by septation, longitudinal and transverse, give rise to the entire fenestration of the pharynx, in Clavelina each primal perforation proliferates a transverse series of stigmata, without first going through the protostigmatic elongation, and then each row of stigmata divides into two rows. At this stage the Clavelina pharynx possesses four rows of stigmata derived from two initial perforations. Are these rows homologous with the four rows in Ciona which are produced from the homologous pairs

TEXT-FIG. 2.



Showing the modes of development of the rows of stigmata in Ascidians. *a*, Phlebobranchia (Ciona, &c.) and Molgulids. A hemitreme divides into two protostigmata ventrally and by synapticulation forms two rows. *b*, Stolidobranchia (except Molgulids). The protostigmata arise separately, each producing one row. *c*, Haplobranchia (Clavelina, &c.). The stigmata themselves arise separately, except the first four of a row in some cases.

of perforations ? I think the answer must undoubtedly be in the negative, for the rows of stigmata in Ciona also possess the power of dividing into two, yet it is not by this process that the rows in question are produced. It follows that each primary perforation in Clavelina, while genetically homologous with the corresponding one in Ciona, is actually equivalent only to a protostigma. Potentially it is a gill-slit, but de facto it is only a half gill-slit ; and it never becomes more, because the physiological precocity which characterizes it has rendered morphological completion unnecessary, if not impossible. In Distaplia this precocity is still greater, for each of the primary perforations divides into two, one behind the other, as soon as produced, so that the four rows of stigmata are proliferated from the beginning (Julin, l. c., figs. 30–2). This is not Julin's interpretation, it is true, for he regards the precocious division of the larval perforations as equivalent to the splitting of a hemitreme into two protostigmata ; but this theory is at variance with the morphological fact that division into protostigmata takes place at the ventral end of the rows of stigmata (i. e. at the point where tongue-bar and ventral wall fuse in Amphioxus), not at the dorsal end as in Distaplia ; and if all the circumstances, especially the extra yolk in the Distaplia egg, are taken into account, the interpretation I suggest will probably commend itself as correct. It agrees essentially with the opinions expressed by Damas (1904) after a careful study of these problems from a different standpoint. It is in any case a good example of the arbitrary nature of morphological interpretation in this section, where the morphological units have lost their original boundaries and acquired new physiological powers.

By means of this precocity of proliferation the pharynx of Clavelina is able to keep pace with the growth of the atrium without ever completing the form of its two potential gill-slits. So when Julin contends that the Clavelina condition with two pairs of gill-slits is more primitive than the Molgula or Ciona condition with three pairs, I cannot assent to the proposition on these grounds. It is more probable that the primitive

Ascidian pharynx possessed three pairs of tongue-barred synapticulate gill-slits, one of which has been lost in the 'Krikobranchiate' series in consequence of an increase of yolk in the egg and precocious subdivision of the series of stigmata formed from the first two perforations. The only ground I can admit for the primitive nature of two pairs of gill-slits is the fact that in Perophora only two pairs of hemitremes are developed; but these hemitremes divide so precociously that true protostigmata are never developed from them, and it must for the present remain a doubtful point whether Perophora really retains the primitive number or has undergone a secondary reduction. The internal armature of the pharynx in Perophora is very variable, a feature which is more characteristic of degenerate than of progressive types.

Thus the Ascidians show clear signs that their gill-slits have been derived from an Amphioxus-like condition, and not from one resembling that of Appendicularians. But the resemblance to Amphioxus ceases with the attainment of three pairs (=six pairs of protostigmata), and all further fenestration is accomplished by subdivision of the rows of stigmata into which these protostigmata become converted. Clearly the tendency to indefinite multiplication of gill-slits is a peculiarity of Amphioxus which Ascidians never inherited: the divergence of one stock from the other must have taken place when the gill-slits were limited to three pairs, or even less, and had not yet acquired the power of proliferation by division.

These considerations fix an important detail in the organization of the primitive Protochordate: its pharynx was provided with not less than two pairs, nor more than three pairs, of tongue-barred synapticulate gill-slits. This is just the organization that can be correlated physiologically with a change from lophophoral to endopharyngeal methods of food collection in a series of small sessile animals. In Cephalodiscus the lophophoral method is combined with a single pair of exhalant water-canals, but from such a condition the change could not be accomplished simply by dropping a tongue-bar into the canals and cutting off the tentacles. An animal of the same size, collecting the same amount of food by endopharyngeal means, would require to pass at least ten times as much water through its pharynx as does Cephalodiscus, so that an increase in the number of canals would seem essential before the lophophore could be entirely replaced. The current would become increasingly dependent on a powerful ciliation of the internal orifices, and this power would be secured without waste of food by down-growth of tongue-bars as the orifices themselves stretched across the pharyngeal wall. In its individual development of course the early Protochordate probably began its life with one pair, and added the second and third pairs as it grew in size.

We can now return to the question of the atrium. As the original Protochordates were polytrematic, and their gill-slits elongated and synapticulate, the perforations themselves could not have been freely exposed, or the pharynx would soon have been torn to shreds. Since in the embryonic development of Ascidians the first rudiments of the gill-slits appear as pharyngeal pouches, or as proliferations recalling pouches which fuse with the atrial epithelium directly, and without the formation of corresponding ectodermal 'pockets' (Julin, 1904, fig. 7; cf. Delsman, 1912, on Oikopleura), it would seem probable that the original mode of development was similar to that seen in Balanoglossus to-day, and that the tongue-bar grew downwards at the pharyngeal end of the trematic pouch, the ciliated slit being thus protected within the endodermal pouch itself (cf. MacBride, 1894, p. 411). But in various burrowing Enteropneusts the outer pores of the gill-pouches may come to lie in a pair of dorso-lateral 'branchial grooves' (cf. Willey's Spengelia, 1898, Pl. 47, fig. 1), and in others, e.g. Ptychodera, lateral flaps of the body-wall beneath the gill-pores may arch over the back of the body and thus define a functional peribranchial cavity (Spengel, 1893; Willey, 1897, p. 179, Pl. 5). Under the latter circumstances the protection afforded by the distal chambers of the trematic pouches is no longer required, and the U-shaped slits open directly to the surface under cover of the peribranchial flaps (=' genital pleurae'), almost exactly as the shallow gill-slits of Amphioxus and Ascidians open into their respective ' atria '.

Thus, independently of the evidence (whatever its value) afforded by the atrial grooves of the larval Clavelina, a study of the gill-slits themselves points strongly to the conclusion that Willey was right when he derived the lateral atria of Ascidians from primitive ' branchial grooves ' into which the original series of gill-slits opened ; and that he was wrong in deserting this idea and adopting the orthodox Appendicularian theory of ancestry. Shallow gill-slits, like those of Amphioxus and Ascidians, are not primitive structures, even when of the simplest circular form. They possess an inherent tendency to become tongue-barred and synapticulate, a condition which cannot have arisen except under cover. They are therefore to be derived from the complex endodermal funnels exemplified in Balanoglossus, and acquired their shallow form subsequently under cover of protective atrial chambers.

From this point of view, therefore, Ascidians in their development preserve the phyletic order more faithfully than Amphioxus, which flagrantly displays a whole series of large shallow slits without an atrial cover. It is significant, however, that while these primary slits of Amphioxus do not become tongue-barred until the end of the larval growth-period, the secondary slits, which arise under complete cover, are tongue-barred almost from the start (Willey, 1894, figs. 77, 80), thus closely conforming to the tendency in Ascidians.

That Amphioxus larvae should be able to do with impunity what we have assumed was impossible for their ancestors is accounted for by the changed conditions of their development, as discussed more fully below (p. 147). Here it is sufficient to note that the single series of gill-slits, which is abnormal in its development and in its relation to the atrium, is also highly specialized. The slits are arrested in an embryonic condition, enormously dilated, and provided with special sphincters (Goldschmidt, 1905, Taf. IV, V ; Van Wijhe, 1914, Pl. II), which doubtless regulate the flow of water through them.

These peculiarities are emphatically not primitive: they disappear at the metamorphosis and do not recur, even as transitory features, in the formation of the subsequent right-sided series (Van Wijhe, l. c., pp. 11, 16, 36, 53). They are adaptive to the exigencies of a precocious larval feeding mechanism, the significance of which can best be appreciated when the abnormalities of the larval stage are considered as a whole (p. 147). In the meantime I need only recall that in a relatively primitive larva, Tornaria, the U-shaped gill-slit is developed almost as rapidly as in Ascidians (Agassiz, 1873, Pl. II; Morgan, 1891, 1894); so that it is not the precocious and specialized left gill-slits of Amphioxus, but the retarded and unspecialized right slits, which adhere to the primitive mode of development, and this takes place under cover of the atrium.

Thus, on our second point, we reach the conclusion that in Ascidians the development of the atrium before the gill-slits is much more closely in accord with the probable course of phylogeny than is the developmental sequence of Amphioxus, since the common ancestor of these types must already have possessed an atrium before its tongue-barred, synapticulate gill-slits could have assumed their shallow form. As the gill-slits of Protochordata normally assume this form from the beginning of ontogeny, the atrium should already be in existence.

The original atrium must have been paired with lateral apertures, as in Ascidian tadpoles, though no existing animal is known to retain this type unmodified in the adult condition. In Ascidians the two apertures are united during the metamorphosis by the depression of the dorsal surface to form a cloaca; in Amphioxus, owing to precocious development and dislocation of the gill-slits, the original paired formation is precluded, and a single atrium is produced from a median ventral groove (Lankester and Willey, 1890, especially p. 456; cf. MacBride on 'atrial folds', 1898, 1900; Lankester, 1898; Smith and Newth, 1917). The causes of this divergent procedure are not far to seek, if we assume a common starting-point in an ancestor provided with lateral atria which developed ontogenetically from a pair of 'atrial grooves' (cf. Willey, 1893, figs. 5, 6). With the great development of the dorsal musculature along the Amphioxus line, the embryonic grooves would be driven more and more ventrally until, on the development of asymmetry, if not before, the dislocation of the left series of gill-slits to the mid-ventral line would render a ventral fusion of the two grooves inevitable (cf. development of atria in Botryllus from a mid-dorsal involution, without any ontogenetic trace of the phyletic migration).

In Balanoglossus, moreover, it is a point of great interest that in the development of certain species the first two gill-slits, together with the collar-pore, open on each side into a definite ectodermal depression or chamber, which is closely comparable with the larval atrium of an Ascidian. This was described by Morgan in his account of the metamorphosis of the large West Indian Tornaria (1894, pp. 51, 66, figs. 67–9), which has been referred by Stiasny (1927) to a species of Ptychodera. It is therefore highly probable that these larval chambers become produced and modified, as development proceeds, to give rise to the cavities, already mentioned, which in the adult are bounded by the ‘ genital wings ’.

The third point in which Ascidian development differs from that of Amphioxus and the Vertebrates, viz. the independence of the larval and adult nervous systems, raises deep issues. The conservatism of the nervous system is almost axiomatic in morphology, and the test of innervation one of the surest guides in the determination of homologies. If it can be shown that in Ascidians the larval nervous system contributes nothing to the adult organization, we shall have come upon a crucial fact of great significance for the interpretation of Ascidian development and evolution.

In other cases of profound metamorphosis (e.g. barnacles, Diptera, &c.), however great the destruction of larval tissues, the central nervous system remains intact, i. e. it is laid down in the embryo, and survives all the changes of form which go on in the body, subject only to minor changes in the degree of concentration. On the other hand, in the metamorphosis of an Ascidian, apart from the absorption of the purely locomotive tail, with its muscles and notochord, there are no radical changes in the visceral organization. The atrial cavities enlarge, the stigmata multiply, the digestive organs differentiate, but all the changes are of a simple and progressive character on the basis of essential features already established in the larva, and are far slighter in character and extent than those involved in the metamorphosis of one of the higher Insects. Yet in spite of this continuity of the visceral organization, the continuity of the nervous system is completely snapped. The whole of the larval nervous system—brain, sense-organs, and nerve-cord—is destroyed; a new brain is developed ab initio; and new nerves grow out from the new brain to supply the various parts of the body. The process is not one of simple obliteration of the neural canal, of atrophy of those parts connected with locomotion, and survival of those which control the viscera. This is an erroneous statement commonly repeated in the text-books, and the error is perpetuated by the unfortunate name 'visceral ganglion' which has gained currency in lieu of 'brain' for the principal nerve-centre of the larval Ascidian. Yet, as shown by Salensky (1893), and confirmed by Grave and Woodbridge (1924), this so-called visceral ganglion gives off no nerves to the viscera at all, and is simply and solely the co-ordinating centre by which the cerebral sense-organs of the larva are connected with the adhesive papillae in front and the caudal muscles behind. Our own observations on Botrylloides are in entire agreement with this account, and we have seen no need to supplement Grave and Woodbridge's detailed figures of the similar conditions in Botryllus.

This larval nervous system is entirely destroyed and removed by phagocytes at the metamorphosis, and the whole of the adult nervous system is a new development proliferated from a minute remnant of the embryonic neural canal. The region in which this proliferation takes place is usually included in the neurohypophysial canal, immediately behind the sensory vesicle (Botrylloides, Pl. II, figs. 6, 7; Ciona, Willey, 1893, fig. 12; Distaplia, Salensky, 1893, Tab. V, fig. 8), and, conformably with this, was assigned to their cul-de-sac cérébral in Clavelina by Van Beneden and Julin (1887). In this form, however, Willey figures the rudiment as arising from the wall of the sensory vesicle after separation of the neurohypophysial canal (l. c., fig. 44), a discrepancy which is not met by his remark that brain and canal both 'proceed from the same epithelial tract' (p. 314). It would seem probable that the real point of origin of the brain rudiment lay a section or two farther back in Willey's preparations, in that 'hinder region of the cerebral vesicle' where the 'boundary line between the hypophysis and the developing ganglion was by no means so distinct as in fig. 44' (l. c., p. 313). From the existence of an area of continuity ('fusion' in the language of Metcalfe, 1900, p. 507) between the adult brain and neural gland in Clavelina, it is practically certain that in this type, as in all others investigated, the rudiment of the adult brain is bound up with the hypophysial tube, and not with the larval sense-vesicle.

This segregation in space and time between the larval and adult brains applies equally to the posterior extension of the nervous system described in various adult types of Ascidian—the 'cordon ganglionnaire viscéral' of Van Beneden and Julin, the 'Ganglionzellstrang' of Seeliger. According to Metcalfe (1900) this dorsal or 'visceral' nerve-cord, which is absent altogether in Botryllus and many simple Ascidians, and has such varied and confused relations with posterior extensions of the neural-gland system, is anatomically an extension of the adult brain in Cynthiids and Molgulids.[1] In Clavelina, the only form in which its development has been investigated, Van Beneden and Julin established its independence of the actual nervous system of the larva, and its development at the time of metamorphosis in continuity with the adult brain. It does not represent a persistence of any part of the larval visceral ganglion, as stated by Sedgwick (l. c., p. 23) and others, but develops separately, exactly like the adult brain, by transformation of the epithelial lining of a portion of the neural canal. This lining is continuous with that of the cul-de-sac cérébral (=Willey's neurohypophysial canal) from which the adult brain

[1] Brien (1927) seems inclined to doubt this continuity in any Ascidian.

develops, and the two form together a single unit of structure which can only be regarded, as all investigators since Van Beneden's time have in fact regarded it, as a persistent part of the neural canal of the embryo, from which the sensory vesicle and 'visceral ganglion' of the larva have been, in a sense, precociously segregated. At the metamorphosis, the epithelial cells of this section of the canal lose, for the first time, their embryonic, undifferentiated character, and become transformed into the solid brain and ganglionated cord of the adult, while the larval brain, sensory vesicle, and caudal nerve disintegrate and disappear.

This independence of the two nervous systems was clearly recognized long ago by Van Beneden and Julin, who drew attention to it in the following words: 'C'est un fait bien remarquable que l'atrophie complète ... de toutes les parties différenciées du système nerveux de la larve, alors que le système nerveux de l'adulte se développe aux dépens de parties restées jusque-là à l'état embryonnaire ou épithélial' (l. c., pp. 353–4).

So also Seeliger (Bronn, p. 836): 'Die Zellen des Rumpfganglions haben sich allmählich abgelöst und degenerirten, wie es scheint, sämmtlich, ohne sich in Elemente des Ganglionzellstrangs verwandeln zu können.'

Thus, although larval and adult nervous systems are alike traceable to an origin from the neural canal, they are completely independent one of the other, and it is an error to assert that any part of the actual nervous system of the adult has formed a part of the larval nervous system.[1] The neural tube of an Ascidian has no more, and no less, to do with the nervous system, larval or adult, than has the epineural canal of a Holothurian. Equally erroneous are the widespread text-book accounts which represent the larval nervous system as something much more elaborate than that of the 'degenerate' adult, e.g. in this account of the metamorphosis: 'The nervous system dwindles away to a mere ganglion from which a few nerves come off' (Dendy, 'Evolutionary Biology', 1912, p. 401). For in a larva like that of Distaplia, in which, owing to abundance of yolk, the adult characters arise precociously, the two brains can be seen side by side, and from the larval brain not a nerve can be traced except to the sucker and the tail, while nerves to pharynx and viscera, as well as to oral and cloacal siphons, can be seen growing out from the adult brain (Salensky, l. c.).

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[1] For corroboration of this account, and a summary of literature, see Brien's latest and remarkable contribution (1927), from which I quote one sentence: 'le tube neural des larves . . . ne devient pas un élément nerveux chez les Ascidiacés' (pp. 36, 37).

These facts of development seem quite inexplicable on the current theory 'that the tailed larva represents the primitive or ancestral form from which the adult Ascidian has been evolved by degeneration' (Herdman, 1904, p. 62). They are, on the other hand, like many other cases of metamorphosis, perfectly consistent with the theory that in the past history of the group larva and adult have undergone divergent adaptive modifications, with the proviso in this case, that the divergence must be dated back to an epoch before the typical Chordate nervous system had been established.

As the adult solid brain is not a remnant of the larval nervous system, it may be the direct representative of the brain of sedentary metamorphic pre-Tunicate ancestors, which has been preserved, generation by generation, to function during the adult stage, by a kind of 'imaginal disc', exactly as the legs of a fly are preserved in a dormant condition for adult development during the legless early stages of the insect's life.

Thus on each of the three points which were selected for preliminary examination we have reached the conclusion that the current view of Tunicate ancestry breaks down. The absence of coelomic segmentation cannot be explained as a consequence of reduction of size, and the detailed studies made by Seeliger and Ihle of the neuromuscular relations in the tail of Ascidian larvae and Appendicularians suggest incipient, but not vestigial, metamerism (cf. § 4, p. 93). The peculiarities of the Ascidian pharynx are admittedly not primitive, and are traceable to modifications of a limited number of tongue-barred gill-slits (two to three pairs) similar to those of Amphioxus; but those were plainly derived from gill-pouches of the Balanoglossus type, and were converted into shallow slits after the development of a pair of lateral atrial chambers. Hence the precocious development and multiplication of the slits in Amphioxus, together with the form and mode of development of its atrium, are secondary modifications, and Ascidians preserve more primitive relations in these respects. Finally, the discontinuity between the larval and adult nervous systems in Ascidians is too profound to be compatible with derivation from an Amphioxus-like ancestry, and is intelligible only by descent from ancestors with a long history of metamorphosis behind them.

On the other hand, all these points are consistent with the alternative view that the Chordata are derivable from a line of fixed ancestors leading directly from primitive Pterobranchia to Tunicata. A small pharynx of two to three pairs of tongue-barred gill-pouches may well have succeeded the Cephalodiscus type, and so have initiated the various lines which have culminated in Balanoglossus, Amphioxus, and the Ascidians. The primitive stock which first discarded the lophophore is now, so far as we know, extinct; but the Ascidians, with their habits of fixation and budding, may be regarded as the most direct descendants of the Pterobranchia, since their pharynx, highly specialized as it now is, contains the equivalents of not more than three pairs of gill-clefts. Balanoglossus and Amphioxus are explicable on this theory as derived from the sequence at different evolutional levels by loss of fixation, retention of larval symmetry, and independent adaptation to a burrowing life, the flexible, elongated body and indefinite multiplication of gill-slits being secondary, but natural developments under such conditions.

The principal difficulty of such a theory is the consequence it imposes of explaining the Ascidian tadpole as an interpolation in the life-history. Some preliminary remarks will therefore be offered on this point before attempting to corroborate the hypothesis in detail.

§ 3. LARVAL EVOLUTION AND THE ORIGIN OF THE ENDOSTYLE.

When Annelids became changed into Molluscs, the Trochosphere was succeeded by the Veliger, which is simply a Trochosphere with the Molluscan characters of shell and foot superadded, and with the circular prototroch produced laterally into a powerful bilobed velum. This velum is plainly no relic of adult ancestry, but a larval development to sustain the weight of the additional structures carried during the pelagic phase. The bigger the velum, the more fully can the adult organization be developed in the larva without jeopardizing the larval function of distribution. Sooner or later, however, the limit of equilibrium is exceeded; the larva sinks to the bottom; the velum is absorbed, and the snail, already equipped with its essential organs, is ready at once to pursue its particular career.

In the case of a starfish the divergence between larva and adult is greater, and the metamorphosis more profound. In most cases, however, the larval means of locomotion is a slender convoluted ciliated band, which is of little use in the later stages of development, so that some species still anchor themselves when undergoing metamorphosis. But in a species of Bipinnaria, whose habits were described by me many years ago (1893), the pelagic period is prolonged by a substitution of muscular flappings of the pre-oral lobe for the primitive ciliary mode, and the developing starfish is easily carried about by the larval movements. Here the analogy with Ascidian metamorphosis is close, for not only does the larva swim by muscular means, but at the metamorphosis a large part of the larval body, including the muscular appendage, ciliated band, and larval nervous system, is thrown off or absorbed, like the muscular tail and entire nervous system of the Ascidian tadpole. The nervous system of the adult starfish, like that of adult Ascidians, is quite independent of the larval nervous system which it succeeds. This latter system in starfish larvae underlies the ciliated band (Semon, 1888). Whether it acquires any control over the locomotive contractions of the pre-oral lobe is not known, but, as the muscles of this appendage are mesenchymatous and lie directly beneath the ectoderm, the possibility of such a connexion is not excluded.

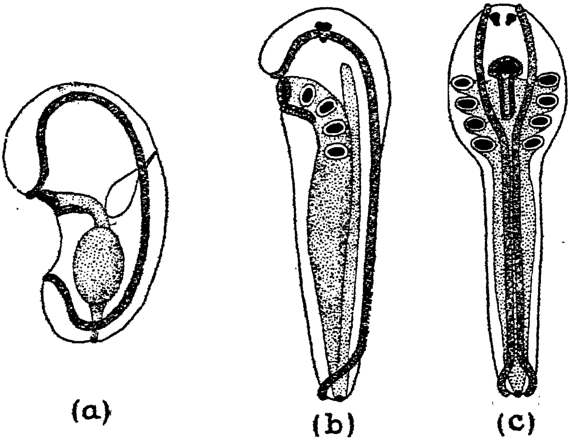
By analysis and analogy we are now in a position to attempt a theory of Ascidian metamorphosis more consistent with the indications of Comparative Anatomy and Bionomics, that the immediate ancestors of the Chordata were benthic animals, collecting microplankton by means of external ciliated tentacles, and of sessile habit. We assume, from the evidence of Comparative Embryology, that the pelagic larvae of these ancestors were simple Dipleurulae, of the Echinoderm-Enteropneust type, provided with apical eye-spots and a circumoral ciliated band.

It is already clear from our present knowledge of Echinoderm and Enteropneust life-histories that the circumoral band is only capable of discharging locomotive functions in the simplest of these larvae (Auricularia) or in their youngest stages (most Holothurians). In other cases it is drawn out into long arms (Plutei), or broken up and rearranged in a series of girdles (Holothurian pupae, possibly Crinoids) and 'epaulettes' (Echinoids), or functionally superseded altogether by an additional girdle or girdles (Tornaria) or by muscular contractions of all the body-processes (Bipinnaria asterigera), or of a special pre-oral appendage (Bipinnaria, sp.).

Our new theory invokes yet another method of prolonging the pelagic phase, but on the lines of the last example. Instead of adding new and more powerful ciliary mechanisms, the larval body elongated, became increasingly muscular, with an increasing tendency towards a segmental arrangement, in consequence of its resorting to lateral undulations as a means of locomotion. This had the effect of bringing the lateral halves of the circumoral band, and its underlying nerve-tract, into closer parallelism on either side of the mid-dorsal line. Subsequently, as a result of additional yolk in the egg, the early ciliated Dipleurula phase was relegated to the embryonic period, and the larvae were hatched as muscular tadpoles, the circumoral band, with its underlying nervous system and its apical sense-organs, having been rolled up meanwhile beneath the surface as a neural canal.

The result of this process would be to bring together for the first time within a single dorsal epithelial tube the rudiments of two nervous systems—a mid-dorsal adult nerve-centre, and a pair of lateral larval nerve-tracts derived from the circumoral band, and connected with the apical sense-organs. If to these features we add the persistence of the larval adoral band and ventral loop as the beginnings of a peripharyngeal band and endostyle, this muscular Dipleurula, which may be distinguished as a 'Notoneurula', would lack only gill-slits and a notochord to transform it into a regular Chordate tadpole.

TEXT-FIG. 3.



Diagrams illustrating the derivation of a generalized Protochordate larva from an Auricularia (from the originals shown at Oxford in 1894). *a*, Auricularia larva; *b*, side view; and *c*, dorsal view of hypothetical early Protochordate larva. The neural folds (=circumoral band) are represented as incompletely united, and the endostyle, within its marginal bands, as incompletely extended.

At the same time, however, that these changes were taking place in the larval form, the organization of the sessile adult was presumably changing from external tentaculate to internal trematic modes of collecting plankton. If we imagine the pelagic larval life prolonged until the rudiments of the adult gill-slits made their appearance (as happens, e.g. in Tornaria), the various organs which had hitherto had a purely larval value would come into possible working relations with the gill-slits, which hitherto had been organs only of the adult phase. This would be a real moment of creative evolution, and it is in this way, as I shall hope to show, that the typical Chordate combination of characters has come into being. Certain features, especially the gill-slits and the beginnings of a notochord, have had their origin in the adult phase of sessile Pterobranch ancestors, while the endostyle and neural canal are the results of a further development and transformation of early larval characters. The neuromuscular metamerism, which is merely incipient in the Tunicate larva, did not attain its complete expression until, by the abandonment of fixation, the larval type of organization was enabled to persist to maturity (paedomorphosis). That this, in part, has been the history of Appendicularians, and of Amphioxus more fully, will be shown in the sequel.

This outline, while not claiming at this stage to be demonstrative, will show, I think, that there is no inherent impossibility in the thesis I have to sustain, that the Tunicate tadpole may have been an interpolation within the life-history of a line of sedentary organisms, and that it does not necessitate ‘ a former free-swimming ancestor ’ to account for it. The homologies between the Dipleurula larva and the Chordate embryo, which were raised in my Auricularia note of 1894, acquire a fuller significance under this new interpretation, and will prove, I believe, more fruitful of results. In that note it was pointed out for the first time how remarkably similar are the medullary (or neural) folds of Vertebrate embryos and the circumoral band of Echinoderm larvae both in their relation to nervous system and sense-organs as well as to mouth and blastopore, and also how strikingly the combined peripharyngeal bands and endostyle of the Protochordata recall the adoral band of Echinoderm larvae, with its significant loop along the floor of the endodermal oesophagus of the larva (Semon, 1888).

The significance of the first point is discussed in a later section of this essay. On the second point the constitution of the endostyle has immediate bearings upon our problem, and it will be convenient to deal with certain aspects of it at once.

The endostyles of Amphioxus and Ascidians are practically identical in structure and relations except in the following points: (1) In Amphioxus the endostyle is a nearly flat, or slightly concave pad, while in Ascidians its glandular sides are steeply arched and enclose a deep groove; (2) the median tract of flagellated cells is adapted to these conditions; it carries relatively short flagella in Amphioxus, but very long ones, extending above the top of the groove, in Ascidians; (3) there are only two tracts of glandular cells on each side in Amphioxus, but usually three in Ascidians, the upper one in Ascidians usually presenting the largest surface towards the endostyle cavity, the lower two being more compact and wedge-like in transverse section.

In both Ascidians and Amphioxus there is a marginal ciliated band surrounding the whole structure except anteriorly where its right and left limbs diverge into the peripharyngeal bands with which they are continuous. But the relations of the marginal band to the endostyle are not alike in the two cases. In Amphioxus the band is part of the thickened pad itself, and is directly contiguous with the outer glandular tract along its whole length (Willey, 1894, fig. 13, after Lankester). It is also, on its outer side directly continuous with the ciliated epithelium of the branchial bars. But in Ascidians the marginal band, although crowning the right and left ridges of the endostyle, is quite isolated by unciliated tracts on either side, i. e. both from the glandular part of the endostyle itself and from the perforated region of the pharynx (Seeliger in Bronn: Clavelina, fig. 71; Cynthia, fig. 72; Botryllus and Ciona, Taf. XVIII).

Alternative views are possible as to the bearings of these differences on the homology of the two organs in Amphioxus and Ascidians. In the development of Ascidians the first sign of differentiation is a longitudinal constriction of the thickened wall of the groove on each side, which defines the outer glandular zone above and separates it from the ventral half as a whole, so that at this stage the Ascidian larva has a certain resemblance to Amphioxus by possessing only two glandular tracts on either side, separated by a narrow band of ciliated cells (Ihle, 1913, p. 500). But the ventral half is not purely glandular, and invariably proceeds to divide into two more or less compact homogeneous glandular tracts, separated by another band of ciliated cells (cf. Seeliger, l. c., pp. 342, 344), and this ventral half of the endostyle, when so completed, bears by itself an even closer resemblance to the conditions in Amphioxus.

Since the greatest differences between the two types are recognizable in the marginal zone of the endostyle, we are on safer ground, I think, if we homologize the two pairs of glandular tracts in Amphioxus with the two lowest pairs in adult Ascidians, and regard the broad uppermost pair in Ascidians, together with the peculiarities in the marginal bands, as special modifications peculiar to Tunicata. In support of this interpretation may be cited the fact that in the few Ascidians in which only two glandular zones are preserved on either side, it is the upper one which has disappeared (e.g. Distaplia, Lahille, 1890, p. 167, fig. 84), and this condition leads directly to that exhibited by Doliolum (Fol, 1876, p. 232, Taf. VII; Uljanin, 1883, Taf. VI). This identification simplifies the phyletic problem, for the elevation of the sides of the endostyle in Ascidians is clearly a secondary feature to be associated with the development of their accessory endopharyngeal apparatus, on which the transport of food and mucilage to the dorsal groove is largely dependent (Orton, 1913 ; Hecht, 1918 ; i.e. ciliated papillae, internal longitudinal bars, transverse membranes, &c.). The additional (upper) tract of gland-cells, as well as the peculiarities of the marginal band, then fall into line as correlated with these new developments.

It follows from these considerations that Amphioxus, in its endostyle as well as in its gill-slits, retains the fundamental or ancestral structure with fewer modifications than do the

Ascidians, and the remarkable changes which it shows in the development of its endostyle may well be expected to throw light on the original evolution of the organ.

As Willey's well-known investigations showed (1891), the endostyle of Amphioxus arises at a very early stage in front of the first gill-slit as a transverse patch of thickened epithelium (Hatschek's 'Flimmerstreif'). This becomes V-shaped and, after a considerable pause during the process of 'symmetrization', grows backwards in the mid-ventral line between the two rows of gill-slits, with its right and left limbs now closely approximated. The front end of the endostyle, however, retains its bifid or double character up to the stage with eight pairs of gill-slits, when the larva has become practically symmetrical, and the endostyle has extended backwards half-way down the series (l. c., fig. 15). The anterior gap in the endostyle then closes up. From a very early stage the peripharyngeal bands are seen to be 'continuous' with the front ends of the two limbs.

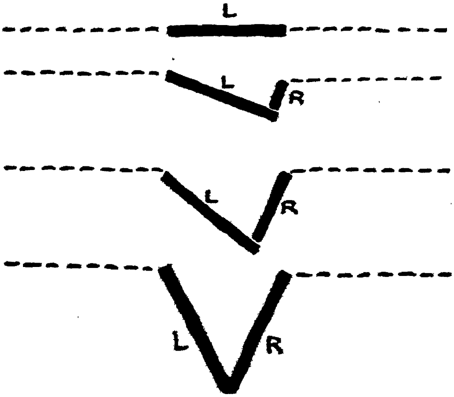
In view of the differences to which I have drawn attention between the endostyles of Amphioxus and Ascidians, a complete histological treatment of the endostyle of Amphioxus during its successive phases of development would be of great value, but Willey's account does not go into details of structure. Two points in particular need further elucidation. What is the precise mode of 'continuity' between the peripharyngeal bands and the limbs of the endostyle? What is the origin of the median tract of flagellate cells? From the examination of several series of sections kindly lent me by Professor Goodrich, I believe that the median tract of flagellate cells is not derived by the fusion of lateral tracts on the inner side of the limbs of the V, but by the incorporation of a narrow median tract of the general pharyngeal epithelium during the process of approximation. A little group of cells arising in this way was readily traceable for some distance in the median line of the 'fused' portion of the endostyle, but I was unable to follow their later history. One of two things must happen: either the pharyngeal cells so enclosed are not really incorporated, but eventually absorbed, in which case the median cells must be differentiated de novo from the fused limbs of the V; or, if incorporated, they have still to undergo modification to transform them into the definitive flagellate cells. Each of these alternatives raises subsidiary questions, but provisionally I assume that the latter process ensues. This assumption leaves only the distinctly paired elements of the endostyle to be derived from the limbs of the V, which are in continuity with the peripharyngeal bands, and the process involved is a simple differentiation into parallel tracts of glandular and shortly ciliated cells.

There are thus three ontogenetic stages in the development of the endostyle of Amphioxus, which, if summed up with due allowance for the more obvious consequences of the temporary asymmetry, are as follows: (1) a peripharyngeal ciliated ring, the ventral portion of which is thickened by the differentiation of parallel tracts of ciliated and glandular cells. This thickened portion runs transversely across the floor of the pharynx inside the mouth, and is the first part to appear; (2) a postero-ventral glandular loop. This—the V-shaped stage—is produced by the bending backwards along the floor of the pharynx of the glandular half of the previous ring; (3) the endostyle proper, which is produced from the previous stage by closure of the anterior gap and differentiation of the enclosed tract of pharyngeal epithelium into the median row of flagellated cells. This integrated organ then grows backwards along the floor of the pharynx (apparently along a predetermined path, see Gibson, 1910, p. 247).

If we interpret this series phyletically, only two stages are really represented, because the glandular tract in Stage I probably owes its transverse position simply to the general suppression of the right-sided organs at this period of development. The transverse patch of cilio-glandular epithelium, which first arises in the floor of the pharynx, consists at the outset entirely of the left limb of the ultimate V-shaped loop, and, as soon as the right limb begins to develop, it immediately pushes the pre-existent transverse limb backwards (see p. 92).

If this interpretation is correct, the glandular tract did not arise phyletically as a differentiation of the lower half of a simple transverse circlet, but by modification of a postero-ventral loop already in existence. The corresponding phyletic stages then become:

(1) A peripharyngeal ciliated band with ventral loop.
(2) A cilio-glandular elaboration of each limb of the loop.
(3) Differentiation of the median area between the two limbs of the loop as a central tract of flagellated cells, and closure of the anterior gap.



It is hardly necessary to point out how closely the first of these stages corresponds with the actual adoral band and loop of Echinoderm larvae, a type of structure which occurs in no other form of animal, adult or larval. In view of the admitted relationship between the two groups, there can be only two theoretical explanations of this correspondence: either Echinoderms are degenerate Chordata, or the endostyle of Protochordata is a further elaboration of the Echinoderm larval loop. The former hypothesis may not be untenable, but I see no way of sustaining it. It is at any rate upon the basis of the second alternative that I proceed.

[*Postscript.*—For some new points in the morphology of the endostyle recently investigated see Garstang and Platt, "On the Asymmetry and Closure of the Endostyle in Cyclosalpa pinnata," 'Proc. Phil. Soc. Leeds,' ii, 1928.]

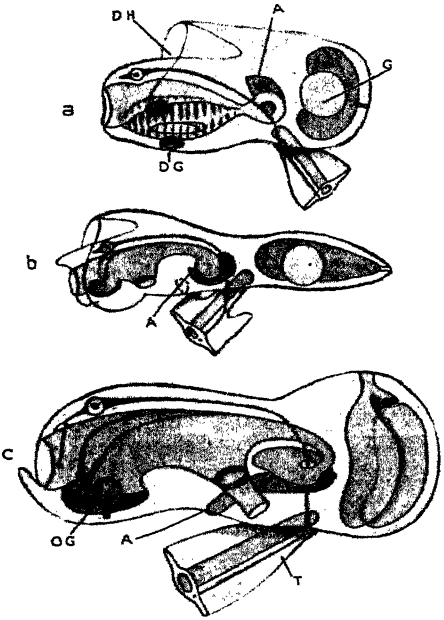
§ 4. APPENDICULARIANS AS PAEDOMORPHIC DOLIOLIDS.

From the standpoint now defined, it is first of all necessary to justify the view already taken that Appendicularians are not primitive Tunicates. We have given one reason for rejecting their claims in this direction, viz. the simplicity of their gill-slits; since, if this is regarded as an ancestral Tunicate feature, the interpretation of the pharynx in terms of tongue-barred slits, like those of Balanoglossus and Amphioxus, becomes impossible. From the high degree to which synapticulation has been carried in Tunicata, it is clearly probable that the Balanoglossus-Amphioxus condition came first, and, with the single exception of the Appendicularians, the various Tunicate conditions are readily derivable from it by processes of further elaboration. How, then, has the Appendicularian condition come about?

The latest summary of the Appendicularian problem is that of Ihle (1913), and it will be convenient to take this as our starting-point. According to Ihle (l. c., p. 518) there are two views to be considered: (1) that Appendicularians are the most primitive of living Tunicates, and (2) that they are neotenic Ascidian larvae. The first view appears to him to be the most correct, although he admits that Appendicularians have been, on the one hand, so specialized for pelagic life and, on the other, so much reduced and simplified 'that in many respects the larvae of Ascidians exhibit more primitive features'. He rejects the neotenic theory because of important differences between Appendicularians and Ascidian larvae, such as the single pair of gill-slits, ventral anus, absence of atrium, &c., and because of the primitive nature of the differentiating characters. He adds from Seeliger (Bronn, pp. 915–16) that there is no group of Ascidian larvae to which the Appendicularians show any special relations.

To these considerations I would reply as follows: (1) the gill-slits of Appendicularians may be primitive only in the sense that they retain embryonic characters (cf. Euherdmania, Ritter, 1903, p. 255, iii); (2) a mid-ventral position of the anus characterizes only the most advanced family of Appendicularians; the intestinal loop of Appendicularians is essentially asymmetrical, with a dextral flexure (the very few exceptions are admittedly not primitive. See Lohmann and Bückmann, 1926, pp. 100, 129); and the anus is definitely right-sided in Kowalevskia and the Fritillariidae, under conditions which are obviously more primitive than in Oikopleuridae (viz. anus behind the gill-slits); and (3) the absence of an atrium may also

TEXT-FIG. 4.



Showing the three chief types of Appendicularian, and the relations of the nerve-cord to the intestinal loop. *a*, Kowalevskia, showing dorsal hood, dextral anus, absence of endostyle, endopharyngeal apparatus, dermal glands; *b*, Fritillaria, showing dorsal hood, dextral anus, elongated genital region, spiracles close behind endostyle; *c*, Oikopleura, showing oral glands, anus in front of spiracles.

A, anus; DG, dermal gland; DH, dorsal hood; G, gonads; OG, oral gland; T, tail, cut across near base, showing notochord, nerve-cord, and horizontal fin-folds.

be a secondary character, as it is in the case of another possible paedomorph, Amphioxides (Goldschmidt, 1905 and 1906). Only the second of these points therefore would be crucial, if true, but it breaks down upon examination. Oddly enough, Ihle himself inadvertently admits it: 'Die Fritillariidae haben die primitive Lage des Enddarms beibehalten. Der After liegt rechts und unmittelbar vor der Schwanzwurzel' (l. c., p. 505). The full significance of this unusual position of the anus will be discussed below.

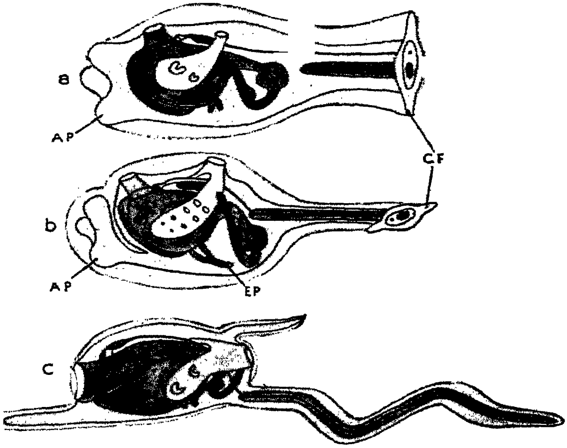
We come then to Seeliger's argument that Appendicularians cannot be regarded as neotenic since there is no group of existing Ascidian larvae to which they can be related. This is a shrewd point, for, as Ascidian larvae never feed, an Appendicularian that fully resembled an Ascidian larva would be unable to live at all! There are, however, two ways out of this difficulty. While it is a fact that the pharynx is never functional during the larval period of any Ascidian, this is due to the liberal amount of yolk with which the egg is provided. But the amount varies between wide limits in different types, so that the larva, on hatching, may be in an early or late stage of visceral development. In a Simple Ascidian of the Ciona type the larva hatches with a pair of open gill-slits; in Molgula or Botryllus the cloaca is developed simultaneously with the atria; in various Synascidians the larva is already a diminutive adult, with larval brain and tail, but with cloaca, fenestrated pharynx, and even a chain of buds all complete; and these differences are mainly dependent on variations in the amount of yolk-provision. Consequently it is possible that Appendicularians may be the modified larvae of some early type of Ascidian the eggs of which were less yolky than those of modern Ascidians, or that they are derivable from one or other of the familiar stocks in which a secondary reduction of yolk has taken place, and the larva has thus become dependent on its own resources for feeding. The first hypothesis would meet the views of those who regard the Appendicularians as primitive, since the larva of a primitive Ascidian would be likely to possess a simpler organization than one of a later type. It would be likely, for example, to possess only a single pair of gill-slits (cf. Euherdmania, Ritter, l. c.), instead of the two pairs with which most modern larvae are provided ; and one of Ihle's difficulties would thereby be removed ('Wären die Appendicularien tatsächlich neotenische Ascidienlarven, dann wäre es nicht einzusehen, warum bei ihnen die Zahl der Kiemenspalten auf ein Paar beschränkt bleibt', l. c., p. 521). This hypothesis, however, cannot easily be tested. The second can be, if we can discover any structural features which plainly point to a relationship with one of the existing types of larva.

The absence of a cerebral eye in Appendicularians may be such a character, since it is also lacking in the larvae of Molgula and Botryllus; but there seems to be nothing else in Appendicularian structure to support a relationship with this group except possibly the indications in Chun's Megalocercus of a rudimentary epineural gland (1887, Taf. V, fig. 4). The significance of this character, however, even in the fixed Ascidians, is still obscure, and for the present no special importance can be attached to it.

The horizontal tail, on the other hand, has bearings on our problem which are fundamental, though hitherto very inadequately appreciated, except by Damas (1904). Even Ihle makes no comments on its significance except as an indication of Appendicularian specialization. Lohmann has so frequently—and rightly—stressed its relations to the working of the 'Haus' that it has apparently come to be regarded as an Appendicularian peculiarity (e.g. 1896, p. 6). Nevertheless, the sinistral twist of the tail into the horizontal plane has long been known as a larval character in Synascidians, temporarily in Clavelina (Seeliger, l. c., p. 774, fig. 162, and Taf. XXVIII), permanently in Distaplia (Damas, 1904) and the Polyclinidae (Grave, 1921). It is only the ventral position of the tail, not its lateral torsion, that is peculiar to Appendicularians, so that a phyletic connexion between Appendicularians and Synascidians is at once suggested. From the nature of the case it is obvious that the condition in Synascidians is more primitive than that in

Appendicularians, since the tail in Synascidian larvae, in spite of the twist, retains its primitive position in continuation of the body-axis, while in Appendicularians, both Oikopleura and Fritillaria, it begins in this condition and then becomes further modified. The tail of the young Appendicularian on hatching is extended behind the body, but is already fully twisted to the left, in both respects resembling the tail of a Distaplia or an Amaroecium larva. Later on, when the genital hump of the Appendicularian develops, the tail is carried to the ventral side and becomes directed obliquely forwards. (For references see Ihle, pp. 517, 518.)

TEXT-FIG. 5.



Showing types of Tunicate larvae. *a*, Cionid Ascidian with vertical tail fin and lateral peribranchial apertures; *b*, Polyclinid Ascidian with horizontal tail fin and median cloacal aperture; *c*, hypothetical larva of primitive Doliolid.
AP, adhesive papilla; CF, cuticular fin; EP, epicardium.

Unless this remarkable twist of the tail has taken place twice independently in the same group, and in the same direction (for in each case the nerve-cord has been carried round to the left side of the tail), it is obvious that Appendicularians have inherited this condition from Ascidian ancestors; and, as simple Ascidians never show this larval twist, we reach the provisional conclusion that the remote ancestors of Appendicularians were not only fixed Ascidians, but compound Ascidians, which multiplied by budding.

Zoologists interested in the inheritance of acquired characters should note, however, that the horizontal tail provides an illustration of this phenomenon exactly comparable with Packard's famous case in Ammonites. When the tail is horizontal in the larva it is derived from an egg in which the tail is coiled vertically (meridionally) around the embryo, while the vertical larval tail is usually coiled horizontally round the equator of the embryo, and thus undergoes no torsion. Clavelina is exceptionally interesting because it connects the two conditions and proves that the horizontal position of the larval tail is an 'impressed character', due to the mode of embryonic coiling. In it the embryonic tail is coiled vertically, and, when hatched, it retains the horizontally twisted tail for some time. Eventually, however, the tail rectifies itself and becomes vertical as in Ciona. On the other hand, Distaplia and the Polyclinids retain the horizontal position throughout their larval life. Here, then, is a clear example of the 'inheritance' of an acquired character. I freely admit, under these circumstances, that the value of the horizontal tail, as a mark of affinity, is debatable.

There are, moreover, several difficulties which militate against the idea that the immediate ancestors of Appendicularians were fixed Ascidians, one of which is the fact that in the latter the intestinal loop is almost invariably twisted to the left, so that the intestine lies on the left side of the oesophagus and stomach, whereas in Appendicularians, even when the anus is median, the general tendency is just the reverse, and in Fritillariidae, as already mentioned, the anus itself is definitely on the right side. The few exceptions to this rule are limited to cases in which the intestinal loop has been secondarily opened out as part of the flotational adaptation of certain types (Lohmann u. Bückmann, 1926, pp. 101, 104). The effect of this dextral torsion of the intestinal loop is seen in the course of the nerve-cord as it passes down the body to the base of the tail.

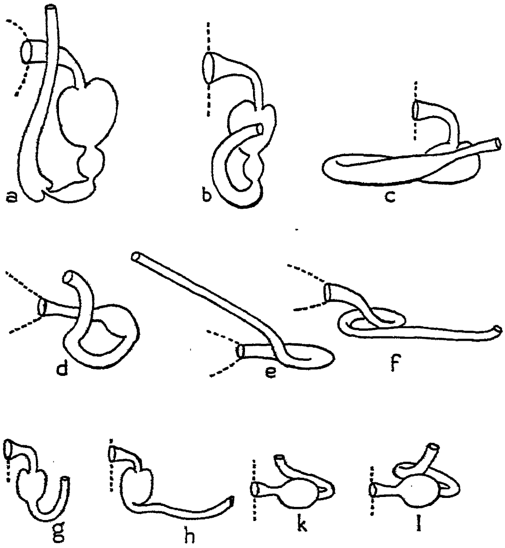
Owing to the deviation of oesophagus to left and intestine to right, the nerve-cord usually runs between the two, i.e. to the right of the oesophagus and to the left of the intestine (cf. Fol, 1872, Pl. iv, fig. 1; Chun, Taf. V, figs. 1 and 4). Only when the loop is very compact, as in Kowalevskia and Fritillaria formica, is this course short-circuited and the nerve-cord reaches the base of the tail by a direct path on the left side of the entire intestinal loop (Fol, l. c., Pl. vii, figs. 1, 2; xi, fig. 1). These various relations are diagrammatically represented in Text-fig. 4.

In Ascidian larvae with a vertical tail, owing to the tail retaining its postero-dorsal position, the nerve-cord is not involved in the intestinal loop; but in Synascidians with a horizontal tail, the nerve-cord, in spite of its rotation to the left, crosses the rectum on the right side of the latter (e.g. Distaplia, Damas, l. c., Pl. xxiii, figs. 12, 13). This, as stated, is the exact opposite of the Appendicularian arrangement. (Cf. Text-figures 4 and 5.)

Examining this matter more closely, we find that, in addition to the Appendicularians, there is only one significant exception to the rule that throughout the Tunicata the intestinal loop, when not secondarily straightened out, displays a sinistral twist. The various types are diagrammatically shown in Text-fig. 6. In all Ascidiacea (apart from Corella and its relatives, which are characterized by a secondary reversal of the normal symmetry, Longchamps, 1900), in Pyrosoma, and in Salpa the intestine is invariably left-sided; but in the oozooids of Doliolum the loop has been straightened out, with the anus posterior, and in the gonozooids either this condition is retained (Doliolum mülleri, Anchinia) or the intestine is retwisted, but now to the right side as in Appendicularians (Doliolum denticulatum, &c.). The condition in the gonozooids is important, because the sexual sterility of the oozooid is admittedly a secondary phenomenon, and in any comparison with Appendicularians we have to take account of the possible effect on the form of the intestinal loop which the absence of gonads may have occasioned in the oozooid.

Here then in Doliolum we have another hint of Appendicularian affinities ; and one which is not inconsistent with the former, since it is commonly admitted that the Thaliacea are derivable from Ascidian ancestors (cf. Neumann, 'Die Pyrosomen der Deutschen Tiefsee-Expedition, 1913, p. 362 ; Korschelt u. Heider, l. c., p. 1420).

TEXT-FIG. 6.



Showing the main types of intestinal loop in Tunicata. *a*, Ascidian ; *b*, Pyrosoma giganteum ; *c*, Pyrosoma spinosum ; *d*, Iasis zonaria (gonozooid) ; *e*, Cyclosalpa (oozooid) ; *f*, Ritteria hexagona (oozooid) ; *g*, Doliolum mülleri (oozooid) and Anchinia (gonozooid) ; *h*, Doliolum denticulatum (oozooid) ; *k*, Doliolum denticulatum (gonozooid) ; *l*, Doliolum gegenbauri, &c. (gonozooid).
A dotted line marks the posterior wall of the pharynx into which the oesophagus opens.

Unfortunately the tail of Doliolum is not functional as a locomotive organ, and its nerve-cord appears to atrophy at a very early stage (Neumann, l. c., p. 106). We can neither assert nor deny that its tail was originally horizontal, as in Synascidians, but a number of striking peculiarities in the tail and other organs corroborate the view of a close relationship with Appendicularians :

(1) The tail, although continuing the axis of the body, is forced downwards by the backward rotation of the cloaca, so that it presents a condition remarkably similar to that of a young Appendicularian (cf. Delsman's figure of the 'larval' Oikopleura in Ihle, l. c., fig. 27, p. 517, with Neumann's figures of Doliolum denticulatum, Taf. I, fig. 5; III, figs. 1, 2, 4).

(2) The nerve-cord, as it passes backwards between the peribranchial involutions, bends down the right side of the future oesophageal region as in Oikopleura (Neumann, Taf. II, figs. 21, 22, copied in Korschelt u. Heider, 1910, fig. 610, A—this being the posterior section, and C the anterior, of the three figured). Neumann, strangely enough, makes no remark on this interesting feature shown by his sections. I infer the bend to be on the right side from his remarks on the peribranchial involutions, p. 109, where the deeper one is described as 'links'.

(3) The single otocyst, developed outside the neural tube, lies on the left side of the brain, as in Appendicularians. In Ascidian larvae the otolith lies on the right side. The homology of these organs may be disputed, but the coincidence is worth noting (see 'The Development of Botrylloides', this journal, vol. 72, p. 34).

(4) The curious ventral proboscis of the larval Doliolum may be represented by the prominent ventral lip of Oikopleurids (Neumann has shown that Uljanin misinterpreted his early larvae, and drew them upside down).

In Appendicularians the shortness of the endostyle, its anterior position, and the presence of a single pair of gill-slits behind it, undoubtedly suggest the retention of very primitive features. This presumption is supported by two considerations which to me at any rate made a strong appeal in the first instance: firstly, the general arrangement is remarkably similar to that presented by the early larva of Amphioxus, if allowance be made for the temporary asymmetry of that animal; and secondly, it accords with the theory which this essay is concerned to defend, viz. that the endostyle was primitively a differentiation of the short ventral loop of the Echinoderm adoral band. We cannot deal more fairly with this idea than to assume it as a working hypothesis, and trace its consequences. It will be admitted, I think, that if the Appendicularian arrangement is truly primitive, it must furnish a possible step in the development of the normal Ascidian arrangement, in which the endostyle is coextensive with the pharynx and the gill-slits lie not behind it, but on either side of it. A comparison of the actual feeding process in an Appendicularian and an Ascidian will best reveal the meaning of these differences and throw light on the evolutional problem.

The Appendicularian, by means of its short anterior endostyle, encircles the mouth with a ring of mucilage. The cilia of the endostyle work forwards, like those of the ventral loop of the adoral band of Echinoderm larvae according to MacBride (1914, p. 463). Those of the peripharyngeal bands work upwards on either side of the mouth, thus maintaining a constant stream of mucus round the entrance to the pharynx. The single pair of ciliated slits in the hinder part of the pharynx gape widely and draw through the mouth a stream of water, which trails the mucilage into tangled strings; other cilia set the whole mass rotating and transform it into a conical sieve which entraps food particles; the twisted apex of mingled slime and food is carried backwards as a continuous cord into the stomach (Fol, 1872, fig. 5).

On the other hand, the adult fixed Ascidian does not make and suspend a sieve within the entrance to its pharynx. It has multiplied and elaborated the pair of simple exhalant tubes of Cephalodiscus into at least four to six rows of minute stigmata, derived by the synapticulation of two to three pairs of U-shaped slits. The wall of the pharynx itself is the filter and requires an endostyle as long as itself that will direct the mucilage upwards and sideways over its fenestrated surface, instead of forwards towards the mouth (Orton, 1913, fig. 7).

Thus although morphologically the simple arrangement of organs in the Appendicularian pharynx appeared to be a possible stage in the development of the Ascidian pharynx, the theory breaks down as soon as the parts are seen at work, and we can understand why no Ascidian goes through an Appendicularian phase in the development of its pharynx. The two mechanisms differ radically in principle; and of the two it is the Ascidian arrangement which is relatively primitive and simple, the Appendicularian that is elaborate and rare. The Ascidian arrangement, by which food particles are entangled and swept together along the walls of the pharynx is merely a variant of the general methods employed by other plankton-feeding organisms (e.g. Pterobranchia, Lamellibranchia, &c.); but the free suspension of a rotating sieve of mucus within the pharyngeal cavity is one of the most delicate and elaborate contrivances for food-prehension in the animal kingdom. The Appendicularian arrangement could no more grow into the Ascidian arrangement while maintaining a functional continuity than can the mouth-parts of a caterpillar into those of the imago. The topographical differences could readily be adjusted by an extension of the endostyle backwards between the gill-slits, and by subsequent multiplication and subdivision of the latter. But the Appendicularian endostyle is specialized to drive its mucus forwards, and the extension backwards of such an organ would be useless. By a multiplication of gill-clefts the inflowing current could be diverted from the axial cavity of the pharynx to its walls; but without a slimy coating on the walls themselves, and a mechanism for renewing it and sweeping it upwards, the net result would simply be to destroy the rotating sieve, and allow the food particles to be carried untrammelled through the gill-slits.

On the other hand, the differences between the Appendicularian and Ascidian types of pharynx reveal all the more clearly the affinities of Appendicularians with Doliolum. Here again we meet with a pharynx in which the perforations are limited to the hinder part of the cavity, behind the endostyle (cf. e.g. Sedgwick, fig. 46; Korschelt and Heider, figs. 767, 835), and the method of feeding is essentially similar to that in Appendicularians, with minor variations associated with differences in the number and obliquity of the row of perforations in different types of zooid (Fol, 1872, fig. 4; 1876, Taf. VII). In each case the endostyle propels its mucus forwards, and as it is swept dorsally along the peripharyngeal bands, 'fringes' of slime are trailed off them by the current, are entangled together on the dorsal side by rotation, and driven backwards as a free cord diagonally through the pharyngeal cavity to the oesophageal aperture. Again, the delicacy of the adjustment of parts to the whole is manifest, and one of Fol's comments may be quoted with advantage: 'Beobachtet man das Thier im Augenblicke wo es eben anfängt Nahrung aufzunehmen [feeding being intermittent], so sieht man . . . dass dieser, am unteren [i.e. morphologically posterior] Ende freie Faden gerade auf den Schlundeingang lossteuert; es muss somit die Richtung der Wasserströmungen dermassen combinirt sein, dass der Faden mit Nothwendigkeit genau den Schlundeingang trifft. Niemals sah ich den neugebildeten Faden etwa durch eine Kiemenspalte austreten' (1876, p. 236).

These remarks, though applicable generally, were made with special reference to the gastrozooid of Doliolum, in which Fol observed that three or four of the anterior gill-slits lashed inwards instead of outwards—a fact of considerable physiological interest and worth noting, even if unique.

Now in the bud-generations of Doliolidae the pharynx is peculiar in possessing a single row of small perforations on each side, set obliquely or transversely to the longitudinal axis. The oblique condition is plainly intermediate between the normal or longitudinal series of perforations shown by Pyrosoma and the transverse series of Anchinia; and the perforations in these types have been generally regarded as having the same morphological value. The view put forward by Lahille (1890, pp. 53–4, 63–6), and subsequently adopted by Julin (1904), that the series in Pyrosoma represents one of the transverse rows of stigmata of an ordinary Ascidian rotated at right angles to its normal position, was shown by me long ago to be completely at variance with the evidence adduced in its support (1892, p. 506; 1893, p. 245), and this view has been corroborated on other grounds by Seeliger (1895) and Damas (1904, p. 780). It follows then, if the perforations in Doliolum are homologous with those of Pyrosoma, that it is the Doliolid series which has undergone rotation. The obliquity of the series in, for example, Doliolum Ehrenbergi marks, as it were, the beginning of an angular rotation backwards through 90° of the whole series of protostigmata, pivoted on a postero-ventral centre near the back of the endostyle; and the rotation may be regarded as completed with the transverse position of the slits in Anchinia.

This theory, unlike Lahille's, seems to be in complete agreement with the facts of the case. It is not the mere shift backwards of the cloacal aperture that has entailed a change in the structure of the pharynx. So long as the lateral peribranchial cavities remained coextensive with the pharynx, the rotation of the cloacal aperture had little effect, as shown by Pyrosoma itself. But in the Doliolids the peribranchial cavities have been withdrawn altogether from the antero-ventral regions of the pharynx, thus preventing the formation of any perforations except in the postero-dorsal region. This withdrawal, in conjunction with the backward shift of the cloaca, is an essential part of their adaptation for muscular propulsion. The peribranchial and cloacal chambers have been merged into one posterior space, and the partition between pharynx and atrio-cloacal chamber has been reduced practically to a transverse or oblique septum across a barrel-shaped cavity.

Thus the position of the gill-slits in Doliolum behind the endostyle is not a primitive character, but emphatically a secondary modification, and we have to face the fact that this is the very feature which we assumed to be primitive in Appendicularians. There can, I think, be no other conclusion than that our assumption was fallacious so far as Tunicata are concerned. The shortness of the endostyle and its position in front of the gill-slits seemed to be primitive features indicative of original larval characters: in reality they are secondary features due to the Appendicularian's retention of the larval form and locomotive tail in combination with an adult Doliolid pharynx, secondarily reduced and simplified.

This interpretation, once reached, is readily confirmed. For, as we have already seen in dealing with the adoral band, the primitive endostyle, as shown by the larva of Amphioxus, was an open loop of the peripharyngeal band, lacking a median tract of flagellated cells, and functioning in all probability simply as a right and left pair of glands to supply the peripharyngeal bands with mucus. It is this organ, and not the definite endostyle, which lies in front of the gill-slits in Amphioxus, and from it the Appendicularian endostyle differs even more deeply than does the normal endostyle of adult Ascidians and Amphioxus. For this organ, as we have seen, is but a further elaboration of the larval peripharyngeal loop; but the Appendicularian endostyle is based not on a simple larval loop, but on the fully integrated adult organ. Its anterior part, which in the larval Amphioxus is an open gap, is here the seat of its greatest complexity. For details see Ihle's special paper on the subject (1907) and his later summary (1913, pp. 496–500).

In Oikopleura, in which type alone its mode of working is reasonably established, the mucus is undoubtedly directed forwards and upwards, as we have seen, but not by the primitive mechanism of the larval Amphioxus. The front end carries a highly specialized tuft of large baling cilia or setae, which are pivoted in front and directed backwards along the floor of the groove. These are plainly the hypertrophied representatives of the median flagella of an ordinary Ascidian endostyle, the rest of the row having been obliterated pari passu with a shortening of the whole organ. A similar remark, mutatis mutandis, can be made with regard to the lateral rows of gland-cells, which in our common Oikopleura dioica are represented by a single cell on each side, as seen in transverse section, in place of the two groups of gland-cells on each side in Amphioxus, or the three of normal Ascidians. Such a condition as this is obviously less primitive than that of Ascidian larvae, and more specialized than that of Ascidian adults. It cannot possibly represent an ancestral stage in the evolution of the organ. As Ihle has remarked : ‘Von dem zwar einfachen, aber doch stark spezialisierten und zellenarmen Endostyl der Appendicularien können wir also keinenfalls den Endostyl der Ascidien ableiten’ (1913, p. 500). From the functional standpoint accordingly the Appendicularian endostyle discharges a simple and primitive function (i. e. supplying mucus to the peripharyngeal band), but does so by the secondary reduction and adaptation of an organ evolved for use under polytrematic conditions (viz. supplying mucus to the pharyngeal walls generally). The larval loop of Amphioxus has only lateral tracts of cilia by which to propel its mucus, in this respect resembling the Echinoderm adoral loop ; the median tuft of cilia of the Appendicularian endostyle represents a later evolutional product altogether, and is additional to the lateral (marginal) bands which still persist. It is neither larval nor primitive, but essentially an adult Ascidian endostyle peculiarly modified and reduced.

The reduction of the endostyle in other species of Oikopleura is not quite so great as in Oikopleura dioica, since in various species examined by Ihle there are two rows of gland-cells on each side separated by a narrow intervening tract of minute ciliated cells. These plainly correspond to the two multicellular tracts of gland-cells on each side of the endostyle of Amphioxus ; but the ordinary Ascidian, as we have seen, possesses three pairs of such glandular tracts, and the same is true of Pyrosoma and most species of Salpa. Here again Doliolum furnishes the connecting link with only two groups of gland-cells on each side (Fol, 1876, Taf. VII, 7 ; Uljanin, 1884, pp. 17, 18, Taf. VI), so that we are able to trace every step in the retrograde evolution of the Appendicularian endostyle from that of the fixed Ascidians by way of Distaplia (see p. 89) and Doliolum.

Moreover, while I have shown how difficult would be a change from the feeding process of an Appendicularian to that of a fixed Ascidian, in consequence of the specialization of the endostyle in the former case, this difficulty does not arise when the evolution from one type to the other is assumed to have taken place in the opposite direction, and Doliolum shows how the change was accomplished.

I must first point out that Orton's admirable study of the feeding mechanism of Amphioxus and the Ascidians (1913, 1914), while making perfectly clear the essential similarity of the process in the two cases, leaves a good deal of uncertainty with regard to the functioning of the various ciliated tracts, especially the action of the marginal bands. It is no easy matter, when a current of water is pouring through a complicated pharynx, to determine the precise influence of different factors in producing the general result. The principal motive-power in these cases is undoubtedly provided by the cilia of the gill-clefts, but the direction taken by the stream within the pharynx is influenced by many other factors, e.g. position of the atrial aperture, ciliation or non-ciliation of the pharyngeal (interserial) bars, &c. Thus in Amphioxus, as shown by Orton himself (l. c., fig. 3), the gill-bars are ciliated on their pharyngeal surfaces, and these cilia lash along the length of the bars from below upwards (in morphological orientation), yet the strings of food particles do not move dorsally in strict parallelism with the direction of the bars, but slide obliquely backwards across successive gill-bars and gill-slits. In this case the main current of water pouring in through the mouth is distributed through the evenly perforated walls and sets from all quarters towards the atriopore on the ventral side. The postero-dorsal direction of the food-strings on each side thus makes an angle with the postero-ventral axis of the water-current, and this resultant deflexion is doubtless due to the action of the frontal cilia described by Orton on the gill-bars, the lashing of which (if truly along the length of the bars) is necessarily antero-dorsal owing to the obliquity of the bars, i.e. the movement of particles on the wall of the pharynx is approximately at right angles to the direction of motion of the frontal cilia.

Now both in Amphioxus and the Ascidians Orton describes the lateral (marginal and intermediate) cilia of the endostyle as lashing mucus transversely from the endostyle on to the sides of the pharynx (pp. 24, 25, 31, 35), and the question arises whether this is strictly correct. Mucus certainly passes sideways from the endostyle to the pharyngeal walls, but this result may be due simply to the indiscriminate centrifugal waving of the long median flagella combined with the general direction of the pharyngeal currents. The median flagella can be imagined as lifting shreds of mucus above the level of the endostyle, and the bipartition of the main current right and left would necessarily carry these shreds on to the pharyngeal surface. But in Amphioxus, without a local current antagonistic to the general backward flow, mucus could hardly be transported at right angles to the long axis of the endostyle, as actually happens. Even if the marginal cilia lash transversely, as Orton claims, the direction taken by particles would be obliquely backwards, as we have seen is the case with particles over the frontal cilia, so that (apart from any contributions of mucus from the peripharyngeal bands) the front part, and especially the antero-dorsal region, of the pharynx would thus receive no mucus and food-collection would there be impossible. On mechanical grounds, therefore, it is probable that the lateral cilia (both marginal and intermediate) actually lash from behind forwards, thus ensuring an even distribution of mucus over the whole side-walls.

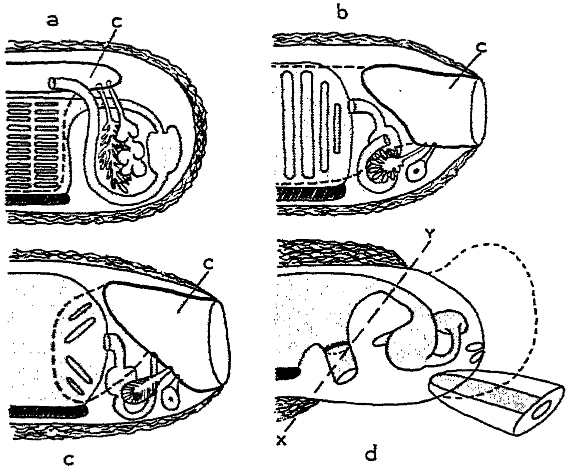
If this criticism should prove to be correct, morphological inference and actual observation would not conflict, as they do at present if Orton's account is final, since theoretically the motion of the lateral cilia on the endostyle of Amphioxus should correspond with that along the peripharyngeal bands, of which the lateral tracts, as we have seen, are simply a ventral continuation.

In Ascidians the probability is even greater, for whereas in Amphioxus the frontal cilia of the gill-bars come close up to the marginal bands of the endostyle, and provide a possible basis for Orton's theory of the direct conveyance of mucus outwards by ciliary action, in Ascidians the gill-bars themselves are not ciliated, and the marginal band is cut off by unciliated pavement epithelium both from the gland-cells of the endostyle and the accessory pharyngeal apparatus which moves the slime-ropes dorsally (see § 3, p. 84). Under these circumstances it is difficult to accept Orton's account of the details of the feeding process. It seems probable that mucus is simply flicked out of the groove by the long median flagella, and carried sideways, not by the marginal bands (which probably work forwards), but by the general set of the water-currents towards the gill-slits and dorsal cloaca. The main function of the lateral bands (both intermediate and marginal) is probably to keep up a pressure of mucus towards the anterior end, and thus compensate for the prevalent backward tendency of the currents in that region.

This digression may be summarized in the remark that while the broad features of the feeding process in Amphioxus and the Ascidians have been made clear by Orton's studies, the precise role of the median flagella and the lateral ciliated tracts of the endostyle remains uncertain. Provisionally it seems probable—in spite of Orton's observations to the contrary—that the marginal bands are not directly concerned in conveying mucus outwards, but forwards; and that the distribution of mucus on to the side-walls of the pharynx is especially the task of the long median flagella in conjunction with the general set of the water-currents.

We can now return to Doliolum as throwing light on the steps by which the Appendicularian feeding mechanism may have been derived from that of fixed Ascidians. Although the gill-slits in the oozöoid are reduced to four pairs of small perforations in a pharyngo-cloacal diaphragm which forms the posterior wall of the pharynx, the endostyle, with its marginal bands, runs along the whole length of the pharynx. If Orton's theory were correct, we should expect mucus to be thrown over the side-walls of the pharynx as in other cases, but we have seen from Fol's careful and circumstantial accounts that this is not the case. It is practically all driven forwards on to the peri-pharyngeal circlet and trailed off from the latter in strings and fringes, not by local ciliary action, but by the force of the inflowing current which sets through the pharyngeal cavity towards the orifices in the posterior diaphragm, where the exhalant cilia are at work. This change is partly due to the concentration of the exhalant apertures posteriorly, and partly to the fact, which Fol repeatedly emphasizes, that the lips of the endostyle are here maintained in close contact all along their length except in front, at the base of the peripharyngeal bands, where the mucus is set free and driven dorsally on each side around the pharynx. Structurally the endostyle of Doliolum is a long open groove as in Ascidians, but, in adaptation to the new conditions, it transforms itself functionally into a closed tube[1] opening only in front—a condition which, it is interesting to recall, led Huxley, during his observations on the 'Rattle-

TEXT-FIG. 7.



Showing stages in the retrograde evolution of the pharynx combined with backward migration and ultimate obliteration of the atrio-cloacal cavity. *a*, Ascidian (Cionid); *b*, Pyrosoma (blastozooid); *c*. Doliolum (oozoid with gonads added); *d*, Appendicularian with dextral anus and rudiments of gonads. *x–y*, the line of division between oikoplastic area in front and genito-spiracular (= atrio-cloacal) area behind. Note opening of anus, gill-slits, and gonads in latter area.

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[1] Cf. Garstang and Platt, 'Proc. Phil. Soc. Leeds,' ii, 1928.

snake ', to describe and name the organ as an axial rod. Plainly the endostyle of Doliolum does not function in the original manner displayed in Amphioxus and the fixed Ascidians, and is already partly degenerate by disappearance of one of the typical pairs of glandular tracts. If the body were to be still further diminished in size, and the protostigmata reduced to one pair instead of four, the endostyle would undergo the further abbreviation which is exhibited by Appendicularians. Thus the rotating sieve of mucus is the end-result of structural changes which can be traced seriatim: (1) backward rotation of the cloaca, withdrawal of the peribranchial cavities, and reduction of gill-slits ; together with (2) functional closure of the endostyle behind, reduction of glandular tracts, abbreviation of the whole organ to a small anterior gland, and suppression of the median flagella except at the anterior extremity.

During the transitional period when the peribranchial cavities were being withdrawn and reduced (probably, of course, through a series of generations), the endostyle was able, by the simple device of opening or closing its groove, to check or increase the lateral or anterior discharge of mucus, as circumstance required. Physiologically, as well as morphologically, therefore, Doliolum furnishes an evolutional bridge from Ascidians to Appendicularians, so far as the essential features of the pharynx and its working are concerned. But there is no passage in the reverse direction.

The agreement between Appendicularians and Doliolids in regard to their pharyngeal mechanism becomes still closer when we distinguish, as we must, between oozooid and blastozooid, and compare the Appendicularian with the former in the earlier stages of its development before budding has begun. In the oozooid of Doliolum (Korschelt and Heider, fig. 832, A) the gill-slits are only four in number on each side, and they undergo no increase either by subdivision or by the addition of new perforations. According to the latest account (Neumann, 1913, pp. 113, 114) these four slits arise in Doliolum denticulatum in rapid succession as independent perforations, the first dorsally, the last ventrally, an order which is in accordance with our theory of the rotation, the dorsal end of the series corresponding with the anterior end in Pyrosoma. These slits accordingly, like those of Pyrosoma, must be interpreted as protostigmata, each corresponding to a transverse row of stigmata in a fixed Ascidian. In most of Neumann's figures (Taf. XIII, fig. 4; XIV, 13; XV, 2), which were drawn from preserved material, these protostigmata appear as a transverse row of equidistant, independent, transversely elongated slits, but in figures drawn from living specimens of the two commonest Mediterranean species, Uljanin (l. c., VII, 11, and XII, 8) represents the slits as arranged in two pairs on each side, one dorsal and one ventral. The two slits of each pair lie parallel with one another, but the axis of the dorsal pair is at right angles with that of the ventral pair. The same arrangement recurs in one of Neumann's own figures (l. c., XV, fig. 1), and also in Grobben's (cf. Korschelt and Heider, fig. 832), so that its constancy cannot be without significance. Uljanin's brief account (l. c., p. 58), while agreeing essentially with that of Neumann as regards the independence of the perforations and the order of development, distinguishes only between the pairs of slits, the dorsal pair arising before the ventral. Each pair, therefore, probably represents a single horseshoe-shaped gill-slit, divided into two equal halves, which arise by independent perforation—a mode of development not known in fixed Ascidians, but intimately related to the second type previously described (Text-fig. 2, *b*).

It follows that the oozooid of Doliolum is a Tunicate provided with only two pairs of primitive gill-slits. Reduce these to one pair, then suppress the tongue-bar formation altogether, and the pharynx of Doliolum would attain the simplicity of an Appendicularian's (cf. Text-fig. 7).

If this condition be imagined in a young Doliolum, in which the persistent tail was still functional (cf. Korschelt and Heider, fig. 767, or Sedgwick, iii, fig. 48, with Text-fig. 5, *c*), and if we further imagine the cloacal invagination, with its shallow atrial wings, to be suppressed altogether (the muscular barrel and a functional tail being inconsistencies), a condition would be produced which would hardly be distinguishable from that of a primitive Appendicularian before the ventral rotation of the tail. As already pointed out (p. 94) the anus would not then occupy a mid-ventral position between and in front of the gill-slits, as in Oikopleura, but a position above the base of the tail, probably on the right side as in the gonozooids of various species of Doliolum, in Fritillaria and Kowalevskia, and the nerve-cord would cross the visceral loop into the tail to the right of the oesophagus (see p. 101) and to the left of the intestine, the tail being in all probability horizontal (Text-fig. 5, *c*).

Is there any evidence that such a process has actually taken place ? I believe there is, and that we have only to consider the problem of the origin of the Appendicularian ' Haus ' to arrive at the same conclusion.

The advocates of the primitive nature of Appendicularians have always kept in the background the fact of the extreme and peculiar differentiation of the Appendicularian integument. Whereas in every other Tunicate the entire surface ectoderm participates in the formation of the superficial tunic or test, in Appendicularians the ectoderm is sharply divisible into an antero-dorsal oikoplastic zone and a large postero-ventral area incapable of test-production. The boundary between the two is a line drawn obliquely across the body from the front of the genital hump dorsally to the region of the endostyle ventrally (see Text-fig. 7, *d*). The contrast between the thick secretory epithelium in front of this line and the thin membrane behind it is exceedingly sharp, and the edge of the oikoplastic zone is often raised into a low ridge encircling the body along this line, from beneath which the genito-spiracular area appears to protrude as a kind of hernia (see especially Uebel's figure of Oikopleura najadis, 1913, p. 626, and various figures of Lohmann's, 1896, Taf. XIII, XV, XVI, XVIII). Into this posterior area devoid of test substance open the spiracles, anus, and gonads. It is in fact morphologically equivalent to a shallow atrio-cloacal chamber, like that of Doliolum, the cavity of which has been suppressed and the epithelial lining everted. As is well known, the epithelium of the atrial and cloacal involutions of Tunicates generally, though derived from the ectoderm, never participates in test-production. Consequently its suppression, in the way I have suggested, would bring to the surface a large posterior area of unsecretory epithelium, together with the apertures of gill-slits, rectum, and gonads, as openings within it (cf. Text-fig. 9).

The boundary line between oikoplastic and non-oikoplastic areas is very commonly the site of some very peculiar outgrowths. In Oikopleurids the ridge already noticed in various species (p. 114) is raised in certain cases (Oikopleura longicauda, Oikopleura cornutogastra) into a thin dorsal fold which extends forwards as a 'veil' over the secretory region (see Text-fig. 9). In Fritillaria the homologue of this 'veil' is present as the well-known dorsal hood (see Text-fig. 4), and functions as a receptacle for the gelatinous nose-bag, which in this genus replaces the 'Haus' of other Appendicularians. Lohmann has shown (1896, Taf. I) that a similar hood also occurs in Kowalevskia, and apparently in Appendicularia (1899, Taf. III, fig. 7), but is so delicate and transparent that it escaped the keen eyes of Fol and previous observers. An ingenious suggestion has been put forward by Lohmann and Bückmann in their recent monograph (1926, pp. 124, 125, figs. 35, 36) to account for the retention and size of this veil in Oikopleura longicauda (=Oikopleura spissa of Fol, and velifera of Langerhans), but it is enough to notice that the presence of this hood or veil in each of the four chief types of Appendicularians, although with three different functions, is clear proof that it is based on a structure which must have been present in the earliest Appendicularians, although originally, in all probability, serving some altogether different purpose. This hood or veil almost certainly represents the dorsal lip of the original cloacal aperture, which in Anchinia is produced into a long tentacle-like outgrowth. This process in the oozooid probably supports the creeping stolon and buds, and is doubtless homologous with the similar outgrowth in Doliolum, although the latter has been shifted forwards from the cloacal margin. It also strikingly recalls the 'atrial languet' of many Synascidians.

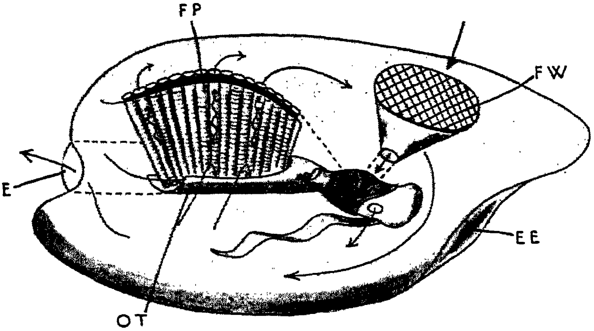
The mid-ventral margin of the original cloacal aperture has apparently left no remnant, and can scarcely be identified with the ventral prominence behind the oikoplast boundary shown in Text-fig. 9, owing to the position of the latter in front of the tail. This prominence, originally discovered by Fol, is only known in the species figured (Oikopleura longicauda), and is of unknown significance (see below, p. 117). In Fritillaria the sides of the hood curve downwards and gradually die away ventrally without definitely uniting with one another. The explanation of this feature becomes clear when the form of the original atrio-cloacal chamber is borne in mind, and the process of obliteration followed in the analogous case afforded by the gastrozooids of Doliolum. In these, as is well known, the atrio-cloacal cavity has opened out almost as completely as I claim it to have done in Appendicularians (cf. diagram in Sedgwick, fig. 52, G; Korschelt and Heider, fig. 831, after Grobben), and the ontogenetic process can be followed in Neumann's admirable account (1913, pp. 201–6, Pl. xxii). The process begins in the cloacal (ventral or anal) region, from which it extends laterally and dorsally into the atrial or branchial region, which is the last to be exposed, as it is the first to be formed. It begins with an elevation of the cloacal floor, and is continued by a gradual widening of the cloacal aperture until the opening is almost coextensive with the area originally enclosed. The median ventral region is completely flattened out, bringing the anus to the surface, and leaving beneath it no trace of a cloacal rim at a stage when there is still a distinct rim around the dorsal and lateral margins (Neumann, Taf. XXII, fig. 7). The base of the Fritillarian hood, and its incompleteness ventrally, thus corresponds closely with the last boundaries of a vestigial cloacal cavity. In the lateral buds of Doliolum there is no tail to complicate the process, and the enlarged aperture retains a simple vertical oval outline; but in a tailed Doliolum a similar process would expose a saddle-shaped area, consisting of a posterior cloacal region above the tail, and a pair of lateral wings extending forwards from it (cf. my diagram, Text-fig. 5 *c*, which does not, however, display the full dorso-ventral dimensions attainable by the atrio-cloacal cavity). Theoretically a mid-ventral strip of test-surface should persist between the spiracles of an Appendicularian to connect the general oikoplastic area in front of the hood with the base of the tail, but the maintenance of an isolated strip of test in such a position would be useless in itself, and would obviously destroy the possibility of an architectural continuity between test and 'Haus', which will next be dealt with. It is in fact along this median tract that the anus must have migrated forwards from the right side of the tail to attain the position which it occupies in Oikopleura. It is just possible that the peculiar lyrate ventral prominence in Oikopleura longicauda already referred to (Text-fig. 9) may be an incidental result of this migration, the anus, metaphorically speaking, having pushed before it a remnant of the ventro-lateral cloacal rim (Fol, Pl. ii, fig. 8).

Having now interpreted the differentiation of the Appendicularian epidermis into oikoplastic and non-oikoplastic areas as the consequence of the opening out of a former atrio-cloacal cavity, I can pass to the subject of the 'Haus' itself, the elucidation of which as an elaborate mechanism for the filtration and capture of 'Nanno-plankton' must always be associated with Lohmann's brilliant and careful researches. With the details and development of this mechanism, however, I only propose to deal in so far as they throw light on the phylogenetic problem. An excellent and well-illustrated summary of Lohmann's work is given by Ihle (l. c., 1913).

Lohmann has shown that, in spite of the complexity of its final structure, the 'Haus' begins as a continuous sheet of cuticular deposit, differing, for example, from that of an Arthropod merely in carrying the differentiation of parts to a further degree. The ventro-lateral wall is almost uniformly gelatinous, but the dorso-lateral wall at the outset is distinguishable into zones of gelatinous material alternating with others which are thin and membranous or deep and laminated.

The conversion of the cuticle into an accessory organ by means of this differentiation and by subsequent foldings and readjustment of parts to one another does not qualify the fact that at first it forms a continuous mosaic of cuticular products resting on a continuous matrix of diversified oikoplastic epithelium. ‘ Hiernach kann kein Zweifel sein, dass die gesammte Gehäusesubstanz als cuticulare Ausscheidung anzusehen ist ’ (1899, p. 382 ; cf. my Text-fig. 9).

Text-fig. 8.



Showing diagrammatically for Oikopleura albicans the serial correspondence between successive parts of the oikoplastic epithelium and of the parts of the filtering mechanism derived from them.

In the epithelium the circumoral zone, Fol’s oikoplasts, and Eisen’s oikoplasts alternate with zones of ordinary test-making cells, and give rise to oral tube, filter-pipes, and filtering windows in corresponding parts of the house.

The oral tube is formed twice, the first being converted into a circular valve round the exhalant aperture (not shown), the second into the collecting or food-trough (shown in simplified form by reduction of the side-walls).

E, exhalant aperture ; EE, emergency exit (former inhalant aperture) ; FP, filter-pipes ; FW, filtering windows (actual inlets, paired) ; OT, oral tube (collecting trough).

The peculiarities of the Appendicularian cuticle begin with its exuviation, for it is then seen that instead of being simply peeled off and cast aside, it is being converted to something else with a new and different function. The primitive cuticle was a simple exoskeleton, moulded on the body and subservient to it. In the Appendicularian the roles are reversed : the differentiations of the ectoderm are subservient to the formation of the cuticular house, and ecdysis is accompanied by so many modifications that it is merely preliminary to a process of functional deployment. 'Die Trennung der Anlage von den Oikoplasten ist nichts anderes als eine Häutung. . . . Die Entfaltung aber hat, so weit mir bekannt, sonst im ganzen Tierreich kein Analogon' (l. c., p. 383).

With the essential nature of the Appendicularian house thus defined as a single, but differentiated, cuticular envelope, the next problem is clearly to determine, if possible, the original form of that envelope by a comparison of the various types. Here we are at once confronted with the fact that the structure, development and mode of functioning of the house is adequately known only in the case of Oikopleura. In his original account (1899) Lohmann cautiously limited himself to the statement that the vesicle of Fritillaria 'stands lowest' in the series, that the 'simple' houses of Kowalevskia and Appendicularia come next, and that the elaborate houses of Oikopleura furnish the climax; but until the first three of these types are known as accurately as the last, it must remain quite problematic whether their simplicity is real or superficial, primary or secondary. The matter can only be provisionally settled by appeal to isolated features and indirect evidence.

In a later account (1909) based on essentially the same facts of structure and development as his earlier account, Lohmann's standpoint shows an appreciable change. The emphasis shifts from the cuticular nature of a 'Haus' subservient to protection and locomotion as well as nutrition, and is concentrated on its significance as a 'Fangapparat': 'So tritt vor allem hervor, wie die Bildung eines Fangapparates den Ausgangspunkt und sogleich den Kernpunkt derselben bildet' (1909, p. 217). The Stufenfolge of his previous account is now definitely put forward as a phyletic succession based on this idea. The gelatinous vesicle suspended in front of the mouth of Fritillaria is regarded as equivalent not to the whole 'Haus' of Oikopleura, but to its 'Fangapparat' alone, the

'Haus' having arisen later 'durch weitere Komplikationen' of its enveloping jelly. The houses of Kowalevskia and Appendicularia are interpreted in the same way, but remain at a lower stage of evolution than the house of Oikopleura. In them the 'Fangapparat' is regarded as having undergone a 'glockenförmig' expansion, in the centre of which the Appendicularian is suspended like the clapper of a bell, not behind it as in Fritillaria and Oikopleura.

Finally, in their recent report (1926), Lohmann and Bückmann have completed this change of front by attaching a surprising significance to certain peculiarities in Tectillaria fertilis (the Fritillaria fertilis of Lohmann's earlier description, 1896). This is a form in which the dorsal hood is produced in front of the mouth by a forward shift of its base, so that the oikoplastic epithelium, which in other forms is restricted to the floor of the hood-cavity, is carried upwards on to its roof (l. c., fig. 46). They suggest, with a somewhat formal reservation, that this may be the primitive condition of the oikoplastic epithelium, i.e. the lining of a more or less tubular multicellular gland, originally limited to the head region. On such a theory the evolution of the oikoplast area has consisted in the gradual opening out of the gland, and the extension of its cells over an increasing area of the dorsal and lateral surface of the body, culminating in Oikopleura in the maximum extension of the secretory epithelium and the disappearance of the hood altogether. It gives circumstantial detail to Lohmann's later view, which has been endorsed by Ihle (l. c., p. 472), that the 'Haus' began as a mere food-trap over the mouth, its enlargement into a complete envelope being a secondary and later development.

Quite apart from the difficulty of comprehending how the filtering capsule could begin in the first instance as the secretion of a gland, or be transformed subsequently into an enveloping house, this theory seems to me to invert the natural order of events altogether. It marks a complete withdrawal from the point of view hitherto consistently advocated by Lohmann himself, according to which the formation of the house was essentially a 'Häutungsprozess' or ecdysis—a view which I venture to submit is still the correct one.

On the latter point, in view of my preliminary remarks, I need only refer again to Lohmann's own arguments and discoveries (1889, p. 207; 1899, p. 382; 1905, p. 357; 1926, p. 119), which seem to me unanswerable; but against the case presented for the primitive nature of the vesicle of Fritillaria additional objections can be urged on many points.

(1) In histological characters Fritillaria is even more profoundly reduced and specialized than Oikopleura.

(2) In general structure Fritillaria has admittedly been modified secondarily ('in der höchsten Ausbildung') for a floating life by gelatinization of the blastocele, &c. (Lohmann and Bückmann, 1926, pp. 101, 105), and the characters of the jelly-vesicle have been claimed by Lohmann as 'eine überraschende Anpassung' to the same end (1902, p. 27).

(3) As a cuticular deposit is primarily a clothing of the body (cf. Lohmann, 1909, p. 219: 'Die typische Bedeutung der Cuticula ist ja zweifellos die einer Schutzhülle'), and as the vesicle of Fritillaria is formed from an oikoplastic area homologous with that of other Appendicularians, and in a similar way, its projection in front of the body is clearly a secondary adaptation and not a primitive disposition.

(4) The same conclusion obviously applies to the peculiar elastic mechanism which causes the vesicle to collapse and spring back under its protective hood when not in use.

(5) The general architecture of the vesicle of Fritillaria is more, not less, elaborate than that of the houses of Kowalevskia and Appendicularia. The latter (Appendicularia?) are provided with a single large median aperture which serves both for the entrance and exit of the water circulated; but the vesicle of Fritillaria is admitted to contain two opposite apertures for entrance and exit respectively, as in Oikopleura longicauda, as well as an oral tube (Lohmann, 1909, p. 217).

(6) If the 'Fangapparat' of Fritillaria arose first, and the 'Haus' of Oikopleura is a secondary extension of it,

the relations of the mouth to the 'Fangapparat' should be primary in all cases. Lohmann has shown, however, that the primary oral tube in Oikopleura gives rise to the circular valve round the exhalant (oral) aperture of the 'Haus', and that the connexion of the mouth with the 'Fangapparat' is secondary, although he was unable to determine how it was brought about (1899, pp. 384, 391, 392; Taf. III, fig. 10, *a*, *b*, *c*).

On all these grounds I find it impossible to accept Lohmann's theory of the evolution of the Appendicularian house. It leads only to a tangle of inconsistencies, and is based on the same confusion between simplicity and primitiveness which has characterized the prevalent interpretation of Appendicularians themselves. Lohmann's achievement is to have shown that every kind of Appendicularian house, whether it encloses the body or not, contains an apparatus which subserves the filtration and collection of extremely fine food particles. He has also shown that the mode of formation of this apparatus in Oikopleura 'ist ein über Erwarten komplizierter, und lässt auf eine sehr lange und wechselvolle phylogenetische Geschichte schliessen' (1899, p. 377). To trace all the steps of that 'long and changeful history' needs more knowledge than at present we possess, but Lohmann's own contributions have pointed the way.

Assuming that the 'Haus' with its 'Fangapparat' has been derived by modifications of a pre-existent cuticle, a 'Haus' which encloses the body is more likely to retain primitive arrangements than one which does not, and the vesicle of Fritillaria must be regarded as the end-result of a process of reduction and specialization. The primary functions of protection and locomotion subserved by the house have been gradually discarded, and the secondary feeding functions retained and specialized, in obvious correlation with the adaptation of Fritillaria itself to a passively drifting life.

Strictly speaking, of course, the evolutional process has not been one of cuticular modification, but of the differentiation of the ectodermal matrix. Starting from a homogeneous ectoderm, like that of Ascidians, the primitive condition must have been that of a homogeneous semi-gelatinous investment. The differentiation of a food-trap in the walls of this investment after partial exuviation is the expression of a modification in the structure and activity of certain groups of cells in the antero-dorsal region. But there are details in the complicated development of this apparatus as described by Lohmann which imply that a gelatinous tunic was not only a primitive feature in Appendicularian evolution, but was indispensable as a predecessor of the membranous parts of the food-trap. The parts of this apparatus which ultimately became hollow are at first filled with jelly, so that a gelatinous cuticle is really the basis on which the membranous parts are moulded. This applies to both parts of the 'Fangapparat', viz. the lateral entrance channels,[1] and the paired series of filter-tubes. The former are laid down as a successive pair of flat striated membranes over the anterior crescent of Fol's group of oikoplasts: 'Die Häute sind durch einen Zwischenraum von einander getrennt, der anfangs von gallertiger Masse erfüllt ist, aber später leer erscheint' (1899, p. 374; Taf. II, figs. 6, 7; Taf. III, fig. 3). The latter are formed as a connected series of fibrils, parallel to the surface, by exudation from the three rows of small prismatic cells behind Fol's giant-cells (l. c., Taf. II, figs. 1, 5, *Rsb*); but the exudations continue, so that new series of connected fibrils are pushed up from the matrix beneath the first series and its successors, until they take the form of a tall row of filter-tubes rising vertically or obliquely above the matrix, and corresponding in number with the transverse rows of formative cells (ca. 27) on each side. Lohmann does not say that these tubes individually contain at first a gelatinous core, but the whole series on each side is supported, as it exudes, by a gelatinous mass on its anterior face, secreted by the well-known transverse row of giant-cells: 'An seiner Oberfläche wird eine gänzlich structur- und formlose Masse ausgeschieden, die das Lumen der Flügel [i. e. the 'wings' of filter-tubes] erfüllt und durch ihre allmählige Zunahme die einzelnen Teile des Fangapparates in ihre definitive Lage zu einander bringt. Sie verschwindet später vollkommen' (l. c., pp. 374, 375, Taf. II, figs. 6, 7, 9). Thus the gelatinous exudations of cells in both parts of Fol's area serve as a scaffolding for the deployment of the membranous parts of the 'Reusenapparat', exactly as the notochord serves as a preparatory axis on which the cartilage of the vertebral column is moulded in the development of a Vertebrate. These relations seem to furnish conclusive proof that in the evolution of the Appendicularian house a gelatinous tunic came first and the membranes and fibres of the filtering apparatus are the product of a later differentiation of the epithelium.

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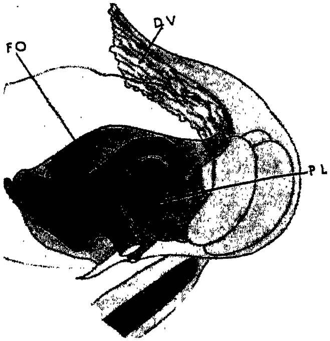
[1] Not to be confused with the 'filtering windows' of the 'Haus'

TEXT-FIG. 9.



Oikopleura longicauda (=Oikopleura spissa of Fol), showing the postero-dorsal veil, Fol's oikoplasts, absence of Eisen's oikoplasts, and the 'house', without filtering windows, in process of deployment from the oikoplastic area (modified from Lohmann).
DV, dorsal veil; FO, Fol's oikoplasts; H, house; PL, posterior limit of oikoplastic epithelium.

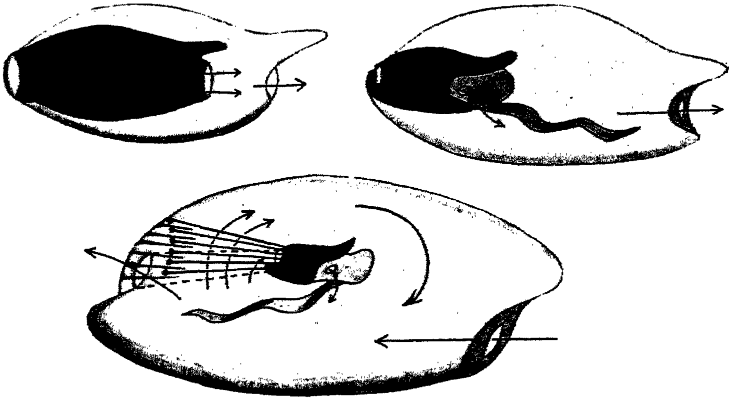
We come back, then, to the problem of the original form of the Appendicularian cuticle, when it was still homogeneous, but with the problem simplified by the elimination of Fritillaria or its 'Fangapparat' from any claim to represent a primitive condition. The radial symmetry of the large house of Kowalevskia is equally anomalous, and is associated with a unique restriction of the oikoplastic epithelium (Lohmann, 1896, Taf. I, figs. 1–3), probably due to the approximation and fusion mid-dorsally of lateral groups of cells corresponding to Fol's oikoplasts. It should probably be regarded, with Fritillaria, as a secondarily simplified form—a conclusion in harmony with the minute size of the animal and with its well-known pharyngeal modifications (loss of endostyle, &c.).

The most primitive Oikopleurid house is admittedly that of Oikopleura longicauda (=spissa of Fol). This house has no ' filtering windows ', and retains two opposite apertures in front and behind for exit and entrance of water respectively. The latter corresponds with the posterior margin of the oikoplastic area ; the former, as already remarked, is connected at first with the mouth of the Appendicularian by the primary oral tube. There can be no doubt that Lohmann is right in regarding the house of other species of Oikopleura as derivable from this type (see Lohmann and Bückmann, 1926, p. 125, fig. 36), since in them the primary inhalant aperture behind is present in a modified condition, closed by membrane, and serving the contained Appendicularian simply as an ' emergency exit ' (Text-fig. 8, EE).

The house of Appendicularia is the only remaining type, but is so imperfectly known that its exact position in the scale can hardly be determined. Lohmann has shown that it contains a paired food-collecting apparatus, remarkably similar at the outset to that of Oikopleura in general appearance (1896, Taf. I, fig. 6 ; 1899, Taf. III, fig. 7), and the single observed aperture of the house almost certainly corresponds with the posterior (inhalant) aperture of Oikopleura longicauda. The apparent absence of a primary oral aperture (exhalant) seems to associate it with Kowalevskia, but it is by no means so certain as in that case that the aperture is really absent. The circumoral tract of the oikoplastic area in Oikopleura, from which the primary oral tube is formed, is greatly reduced in Kowalevskia (Fol, l. c., Pl. x, fig. 5), and Lohmann figures the house arising as a purely dorsal structure (1896, Taf. I, figs. 1–3), which is presumably slipped over the mouth subsequently to its formation. The absence of an oral (anterior) aperture to the house can thus be accounted for, while the large size of the structure when expanded (35×20 mm.), and Fol's experimental study of it, leaves no room for doubt as to the facts. But the house of Appendicularia is the smallest known (2·6 mm.) and direct observation on this point must be wellnigh impossible. The corresponding aperture in Fritillaria would probably still be unknown if Fol had not had the large capsules of living Fritillaria megachile (10×8 mm.) for observation. Appendicularia is in many respects a connecting link between Fritillaria and Oikopleura, and both these genera possess the anterior aperture; the circumoral zone is well developed; and there can be no doubt that the house-rudiment, which Lohmann figured in 1899, approximates much more nearly to the Oikopleurid than to the Kowalevskia type. Thus, if the aperture exists, the architecture of the house must be essentially similar to that of Oikopleura longicauda, even if the filtering mechanism is more simple; and if it does not exist, its absence is almost certainly secondary, and marks a beginning of the series of retrograde modifications which culminate in Kowalevskia.

From this examination it results that in all probability the primitive type of Appendicularian 'Haus', from which all others have been derived either by elaboration or reduction, consists of a hollow, oval or spherical capsule with two apertures at opposite extremities, one oral, and the other formed round the boundary zone of the oikoplastic epithelium, which in primitive cases is limited by the base of a dorsal hood or veil. Assuming this to mark the limits of a former atrio-cloacal cavity, the primitive house must have been a cast-off test, with opposite oral and cloacal apertures. Such a test could only be formed by a pelagic Tunicate of the Doliolid type; and since Doliolum, at least Doliolum mülleri, is known to possess the power of total ecdysis (Uljanin, l. c., p. 14), there is no need to say more than that the case is thus complete. There can be no question of the fact or of the correspondence in essential details. Uljanin observed the process under the microscope under conditions which enabled him to describe it as 'eine vollkommen normale Erscheinung', and to assert 'dass unter der abgeworfenen beschmutzten Cuticula eine sehr dünne neue immer schon ausgeschieden wird'. He points out, moreover, that in Doliolum the test throughout life remains hyaline and structureless, like that of the youngest larval stages of ordinary Ascidians, and contains no cellulose; and that, as in Appendicularians alone among Tunicata, there is no involution of test-substance within the oral aperture (or within the cloacal aperture). These are precisely the conditions required for the beginnings of the evolution of the Appendicularian house.

TEXT-FIG. 10.



To suggest a possible sequence in the early evolution of the Appendicularian 'house' from a Doliolid test—primarily as a flotational device.

It is assumed that the test remained adherent to the mouth after detachment elsewhere; and that, when the tail reversed its direction, this caused complete withdrawal of the Appendicularian within its house, to which it remained attached by gelatinous threads drawn out from the circumoral zone. Two circlets of these threads may have been the predecessors of the primary and secondary oral tubes ultimately developed. Detachment of the first circlet from the body would at once produce a tubular valve around the exhalant aperture, thus setting up conditions of pressure within the house and the beginnings of a circulation.

Development of the filtering mechanism could then proceed.

The modifications introduced into the house for purposes of food-collection form a subsequent story which does not now concern us. I will therefore merely point out that if my criticism of previous views concerning the relations of 'Haus' and 'Fangapparat' is valid, it is in the development of the houses of Oikopleura longicauda and Appendicularia that we may best expect some primitive features by which to interpret the complexities of formation revealed by Lohmann in Oikopleura albicans.

Thus, after a fairly exhaustive survey of Appendicularian peculiarities, there can be no doubt that their simplicity is more apparent than real. When the structure and working of their pharynx and endostyle are examined closely and comparatively, these organs alone yield conclusive proof of a derivation from the more normal Tunicate type. Moreover, in every respect—position and number of gill-slits, structure and functioning of endostyle, form of the intestinal loop, position of the anus, and torsion of nerve-cord—Doliolum has been shown to provide the key to their phylogeny. Finally, the architecture of the house, and its relations to the oikoplastic epithelium, have confirmed our view that the absence of a cloaca and of peribranchial cavities is a secondary phenomenon, and that the ancestor of the Appendicularians was essentially a Doliolum with a barrel-shaped test subject to periodic exuviation. In the dorsal veil or hood of Oikopleura longicauda, Appendicularia, Fritillaria, and Kowalevskia we even seem to have a hypertrophied remnant of the ancestral cloacal aperture with the dorsal outgrowth (=atrial languet ?) that in Doliolids carries the creeping stolon (Anchinia) or its equivalent the succession of migrant buds (Doliolum itself).

The remarkable thing about these features of resemblance to Doliolum is that they are all essentially adult, as distinct from larval, characteristics, and may be thought to give a final refutation of the neotenic theory which I began by defending, since only the tail is left as a relic of larval conditions. As was remarked, however, at an earlier stage (p. 95) Ascidian larvae do not feed, and have no visceral organization of their own distinct from that of the adult phase which, in varying degrees, they seek precociously to develop. The same conditions must have prevailed among primitive Doliolids, before the larva was finally boxed up in an inflated egg-capsule serving as a float. Even if, as I believe, the Doliolids are derivable from some more central stock than the Clavelinid Synascidians, the abandonment of fixation, by obliterating the original boundary between larva and adult, must have facilitated just such a combination of larval and adult characters as we find permanent in Appendicularians. The young nurse-form (oozooid) of Doliolum (Korschelt and Heider, fig. 67; Sedgwick, iii, fig. 48, both after Uljanin) offers a remarkable blend of these characters, and, with certain qualifications, can well be regarded as an Appendicularian in the making. Whether it be called adult precocity, or larval persistence, is immaterial. It is essentially the breakdown of a former separation between the two stages of a metamorphic life-history, and only requires one step further in the process of simplification, viz. the abandonment of budding and consequent reacquisition of sexual powers by the oozooid, to produce a primitive Appendicularian, with larval tail and simplified adult body.

The dorsal hood, in association with the opening of an atrio-cloacal cavity, points probably to the existence in the ancestors of Appendicularians of a dorsal outgrowth, similar to that which in Doliolids is associated with the development of the buds, but it is not in itself conclusive of a former reproduction by budding. In the 'pharyngeal packets' of Fritillaria, however, and the 'oral glands' of Oikopleura we have peculiar organs which seem to be the modified vestiges of the Ascidian epicardium, and thus to complete the story.

Before submitting the evidence under that head, however, which will carry us away from Appendicularians altogether, I must briefly refer to two points in Appendicularian structure which prima facie may seem to conflict with the conclusions already arrived at. These are the differences between Appendicularians and Ascidian tadpoles in regard to the structure of the tail and the arrangement of its muscle-cells.

The tail of Appendicularians is devoid of test-substance, its fin is produced by keel-like extensions of the true cellular wall, and its lateral muscles are provided by a single row of cells on each side of the notochord which, from their large surface and transverse lines of suture, have given rise to the much-discussed appearance of segmentation.

The tail of Ascidian larvae, though similar superficially, is composed quite differently, and consists of two elements, (1) a central axis, circular in section, continuous with the body of the larva, and (2) a tail-fin composed entirely of test-material and continuous with the test covering the body (see my figures 4, T, and 5, CF; cf. Seeliger, 1900, and in Bronn, figs. 173–4, Taf. IV, V; and Martini, 1909, p. 300, figs. 1–7). Although the tail of Doliolum is not functional, and appears to be destitute of test-material, it corresponds with the tail of Ascidian larvae in other respects, being circular in section and provided with several longitudinal rows of muscle-cells on each side, which have the same alternating arrangement as in Ascidian larvae (cf. Uljanin, 1884, Taf. VII, fig. 3; and Neumann, 1913, Taf. XI, XVII; with Seeliger in Bronn, fig. 174).

These differences may again raise the idea of the retention by Appendicularians of a more primitive condition than Doliolum itself affords. But when the special points of resemblance between the Appendicularian tail and that of larval Ascidians are borne in mind, these two points of difference are seen to be of minor significance, and are readily explained as part of the many adaptations which have fitted the Appendicularian tail—whatever its origin—to the specialized functions it discharges in relation to the 'Haus'. As Lohmann has well said (1896, p. 6), 'Beide Merkmale [i.e. tail and house] hängen auf das Engste zusammen und haben sich offenbar gleichzeitig ausgebildet'. These acquired functions consist not only of the creation of currents through the 'Haus' (or into the vesicle, as the case may be) by a mode of undulation recognizably different from that used during free locomotion, but also of manipulative powers, by which the tail assists in extending the house-rudiment over the body and in enlarging and shaping its internal spaces. Clearly a tail which has acquired a mobility and versatility of this order cannot be expected to have retained the primitive features of its origin. Its free articulation with the body, its width, length, and flexibility, its smooth surface, sensitive tip, and specialized neuromuscular apparatus, must all be the result, more or less, of special modifications, whether the ancestor was Amphioxine or Ascidian.

Assuming, as we must, that the Doliolid ancestor possessed a locomotive larva with a tail-fin of test-material like that of Ascidians, it is obvious that a fin of this structure, sufficient for the brief career of a tadpole, would be quite inadequate for a creature that depends lifelong on the efficiency of its tail, especially as the cuticular fin would presumably be shed at every ecdysis and leave its possessor helpless. To substitute for this a dorsal and ventral keel-like extension of the tail itself would be no difficult operation, especially as the opening up of the atrio-cloacal cavity would bring the tail into immediate contact with a tunic-free area, so that a slight change in the cell-mosaic of the gastrula would enable the tail to be clothed by the same tunic-free epithelium as the cloacal area itself.

As regards the arrangement of muscle-cells I can readily agree with Martini (1909, p. 306) that the Appendicularian type is theoretically capable of derivation either from the fully segmented myotomes of Amphioxus or from the unsegmented arrangement of the Tunicate larva, but the question is, which is the simplest hypothesis consistent with the facts? In effect Martini rejects the second alternative by his opposition to the theory of neoteny, but he seems not to have realized that in expounding the first he was merely substituting one larva for another, and that neoteny (in its wide sense) was involved in either case. His proposition is that by ‘ Eutelie ’ the myotomes of Amphioxus may have been reduced in Appendicularians to the form of single cells, by successive stages of reduction. He begins with an Amphioxus embryo, in which the somites are still undivided, and in which the muscle-plate consists only of six cells (fig. 5). By assuming that the loss of a coelom would leave these little groups of cells in situ, and their metameric arrangement intact, it is of course ‘ sehr leicht ’ for

'Eutelie' to convert 'den Querschnitt der Amphioxus-Larve in den der Appendicularie' (fig. 6). But what of the metamerism? It is impossible to admit that the metamerism of the muscle-plates of Amphioxus would survive the loss of somite-formation. As soon as coelom-formation ceased to be a feature of the ancestral development, the Tunicate condition with wandering mesenchyme would be at once established, and an unsegmental arrangement of muscle-cells, as in Ascidian larvae, would follow. On his own theory, therefore, a condition essentially similar to that of Ascidian larvae would necessarily intervene between the ancestral Amphioxus larva and the Appendicularian. Moreover, so far as 'Eutelie' is concerned, it is surely a simpler hypothesis to take the arrangement of muscle-cells actually found in Tunicate larvae as the starting-point, and reduce the number of parallel longitudinal rows from three to one on each side, in order to produce the Appendicularian arrangement.

The histological difference between the muscle-cells of Appendicularians and those of Ascidian larvae is relied upon by Martini in support of his Amphioxus theory, but a last stand can hardly be made upon this. If Appendicularians have been derived from Amphioxus, then Ascidians have been derived from Appendicularians, and the muscle-cells which in the latter have a one-sided arrangement of their fibres must have been converted into those of Ascidian larvae with fibres on all sides. This again is surely the wrong way round. The arrangement of fibres in the Ascidian muscle-cells is simply the generalized, primitive, arrangement characteristic of mesenchymatous muscles everywhere; while that in Appendicularians and Amphioxus is the specialized arrangement associated with expansion over flat surfaces, epithelia, &c. The former is primitive, the latter secondary. In fact the muscles of an Appendicularian, so far from pointing to an origin from fully segmented ancestors, are just the primitive muscles of an Ascidian larva reduced in number and specialized for their peculiar and more extended tasks.

Under the term 'Eutelie', which has been used above, Martini

(1909, 1910) attempted to distinguish a special type of phylogenetic simplification, of which the Appendicularians, in his opinion, furnish one of the chief examples. The other principal illustration is supplied by the Nematoda. Both these groups display phenomena of ' cell-constancy ', i.e. the presence in definite number and position of individual histological units, which may correspond to entire organs in other groups, and are equally characteristic of all the individuals of a species. Associating this phenomenon with the direct or determinate mode of development followed by the same groups, and assuming that it is the end-result of a process of phylogenetic simplification, Martini defines ' Eutelie ' as the accelerated attainment of a biological goal by the utmost simplification of the organization and the most precise developmental method. I use the latter phrase to express the author's ' praeziseste Arbeit ', ' Praezisionsarbeit ', and ' präzisen Entwicklungsmechanismus ', but exactly what Martini means by these peculiar expressions it is not easy to understand. As an equivalent of the first he gives in brackets ' determinierte Entwicklung ' (p. 298), and qualifies this term as being used ' im deskriptiven, nicht experimentellen Sinne ' (p. 292)—by which one is led to understand him to mean simply the elimination of ' palingenetischen Umwegen ', i.e. direct non-recapitulative development (cf. p. 295). As direct development in this sense is equally characteristic of cases hitherto classed under ' Neoteny ', whether total (e.g. Axolotl) or partial (e.g. Perennibranchiate Amphibia), clearly ' Eutelie ' would seem to be distinguishable from the latter merely by the greater extent of the retrogressive process (viz. cell-reduction).

To meet this difficulty Martini adopts two lines of argument: (1) Neoteny, being essentially an arrest of development, either as a whole, or with reference to particular organs, cannot be applied to cases of phylogenetic simplification which retain more primitive features than do the larval forms of their group. As an example of this he gives only the case of the Appendicularian muscles, the weakness of which I have already exposed. Moreover, if the example were valid, it would only tend to show that Appendicularians were neotenic Amphioxi instead of neotenic Ascidians. (2) For a similar reason, the term 'Neoteny' should be restricted to cases of 'total Neoteny', since in such cases there is no doubt that an actual larval form has been arrested in development by precocious maturity. There is something to be said for this proposal of Plate's; but the absence of a name for the large class of 'partial Neotenics' would not remove their existence, or render 'Eutelie' in any sense a suitable substitute.

Martini remarks that 'die partielle Neotenie nur ein Modus eines phylogenetischen Rückbildungsprocesses ist' (p. 296). It must be equally apparent that 'Eutelie' is only a mode of partial Neoteny. Its distinctive features are neither phylogenetic retrogression, nor arrested metamorphosis, nor direct development, all of which it shares with partial Neoteny; but simply the extension of the process of simplification into the cell-constitution of the various organs. As the term 'Eutelie', with its emphasis on 'direct development', 'going straight to an end', is not distinctive, and is applicable to a far wider range of cases than its author intended, it should clearly disappear, and the phenomenon of cell-reduction, which is of great interest and importance, should be distinguished under some such descriptive title as 'Katacytosis', or 'Oligocytosis'.

With Plate and Martini, on the other hand, one can appreciate the advantage of a distinctive term for cases of total Neoteny, which present a special problem, and seem in general to arise abruptly under particular conditions in the course of individual life-histories. Reserving 'Neoteny' for these cases, Giard's 'Progenesis' would cover its extension into still earlier phases of the ontogeny.

When individual larval features alone are retained, however, in combination with a simplification of adult characters, and a readaptation of the whole, we have a different problem, which, as Martini has well emphasized, demands a long phyletic process of gradual change. The process of simplification and adaptation may proceed along different lines in different cases, but all are intelligibly covered by the term 'Paedomorphosis'

(Garstang, 1922), while a strict use of 'partial Neoteny' would leave many significant cases outside the pale (e.g. Holopus).

§ 5. EPICARDIAL VESTIGES IN APPENDICULARIANS AND AMPHIOXUS.

Pharyngeal Packet of Fritillaria.—This structure is a solid group of protoplasmic cells wedged in the floor of the pharynx immediately behind the endostyle, and projecting backwards between the spiracles so as often to fill the space between the pharyngeal wall and the ectoderm in this region: 'Unter Pharyngealpaketen sind eng aneinanderliegende, plasmareiche, grosse Zellen zu verstehen, die sich vom Hinterende des Endostyls nach hinten erstrecken und sich auch der Innenwand der Kiemengänge anlegen können. Die Verbindung mit dem Endostyl ist sehr bezeichnend. Die Bedeutung der Pakete ist nicht bekannt' (Lohmann u. Bückmann, 1926, p. 164, figs. 48, 49. Also cf. Lohmann, 1896, Taf. II, fig. 8; IV, fig. 1; VII, fig. 2).

This mysterious 'packet' finds a ready explanation on the Appendicularian theory here maintained, since from this standpoint it can be nothing but the modified vestige of the Ascidian epicardium. It is clearly no functional organ necessary to the life of Fritillaria, for it varies from species to species in the most extraordinary and erratic fashion, and finally becomes resolved, especially in the haplostoma series, into a loose arrangement of cells varying in number from five or six in Fritillaria drygalski (Lohmann und Bückmann, fig. 53) to three in Fritillaria borealis (Lohmann, 1896, Taf. VIII, fig. 2), or divided into a pair of lateral groups adjoining the inner margin of the spiracles, and undergoing the same diminution in number from groups of two to three cells in Fritillaria helenae (Lohmann und Bückmann, fig. 52) down to a single cell on each side (ibid., fig. 47) or vanishing altogether (Fritillaria haplostoma). A striking feature of the packet, in addition to its relations to the endostyle, is its tendency to an asymmetrical position or development on the left side (Fritillaria venusta, pellucida, helenae—reversed only in Fritillaria scillae).

A good idea of the size of the 'packet' in Fritillaria pellucida is conveyed by a section given by Salensky ('Études', 1904, iii, Pl. xiii, fig. 22), and a full account of it is given by Martini in his cytological study of the same species (1909, pp. 126–9, Taf. II, fig. 35). Ihle and Martini, who confirm its glandular properties, follow Salensky and term it 'branchial gland' ('Kiemendrüse'). In young individuals of this species it consists, according to Martini, of four cells, two large and two small, but the latter tend to atrophy and disappear in later life. Adjacent parts of each cell together constitute a patch which forms the floor of the pharynx between the endostyle and the left spiracle, but the peripheral regions of the gland bulge outwards beneath the basal membrane of the pharynx: 'So resultiert in der Pharynxwand eine zusammenhängende Stelle, die wir als die weite Mündung der Drüse auffassen können' (Martini, p. 129). The function of the gland is quite problematic, although the fact that it secretes fluid droplets into the pharynx is beyond question. Salensky suggested that the secretion might have a food-entangling function, to supplement deficiencies of the small endostyle, a theory to which there are fatal objections, based on the position of the gland, apart from the reason given by Martini for rejecting it.

In considering the homologies of this organ, the special peculiarities of Appendicularian cytology have to be borne in mind, for reduction in size has been accompanied in this group by an extraordinary modification of normal Metazoan structure. Neither cells nor nuclei seem to have the same elementary or unitary values as in ordinary animals: each cell is almost equivalent to a syncytium, but with a single branching and lobulate nucleus, instead of a whole series of normal nuclei inside it. The pharynx, which in a normal Ascidian or its larva is constituted by a regular multicellular epithelium, is here nothing but a basement membrane deposited by a thin sheet of cytoplasm under the control of seven nuclei located at strategic corners. Suppose the ancestor of Appendicularians to have had the constitution of a Synascidian larva, with a pair of epicardial tubes, separate or united distally, what form would these tubes take under the conditions of Appendicularian cytology, especially if we can assume that they were no longer wanted either for budding or for the septation of blood channels? Sooner or later of course they would disappear, as in most Appendicularians apparently is the case, but in the few Fritillarias in which these 'packets' persist, their form, as suppressed diverticula from one or both sides of the pharynx immediately behind the endostyle, and their variability, as vestigial organs, fulfil all the conditions required. The glandular transformations which this modified epicardium has undergone (if the homology be accepted) is probably not a specific functional adaptation, but something more akin to 'fatty degeneration', by which the individual attempts, metaphorically speaking, to get rid of a useless heirloom. A closer parallel is probably to be seen in the conversion of tongue-bars, gill-pouches, and other pharyngeal vestiges into endocrine glands in Vertebrata. In any event a glandular modification of the epicardium in Ascidians is not without precedent, since Ritter has described it (1903) in the remarkable Polyclinid Euherdmania, and under conditions which are at least consistent with a theory of 'glandular degeneration'. Euherdmania is unique in possessing two long, completely separate epicardial tubes, of which the left is feebly developed, though thick-walled and glandular. As the pharyngeal apertures of both tubes are closed, the glandular secretion cannot escape. Nothing is yet known of the details of the budding process, but the glandular tube is almost certainly sterile. From such a modification it is but a further step to the condition realized in Molgulids according to Damas (1902). Here the epicardium has been saved from extinction by taking over a renal function, comparable to that of the renal vesicles in Ascidians. The peculiar relations of the heart to this epicardial kidney, together with special ontogenetic details, render Damas's interpretation irresistible.

The oral glands of Oikopleura are a pair of conspicuous round bodies lying beneath the integument on either side of the endostyle in many species of this genus. They were shown by Fol (l. c., p. 25) to open to the exterior and to secrete a fluorescent (or phosphorescent) material which decorates the surface of the ‘ Haus ’ (cf. Lohmann, 1899, pp. 208, 213 ; Lohmann u. Bückmann, 1926, pp. 111, 115). They seem to be unicellular, with a lobulate or branched nucleus (Lohmann, 1896, p. 18), and I can confirm this, so far as Oikopleura dioica is concerned. Salensky has claimed them to be multinucleate, though cell-outlines are not recognizable in them (1903, p. 6 ; 1904, p. 71). In any case the cell-reduction which Appendicularians have undergone renders the distinction between uni- and multi-cellular of little moment : the predecessors of these glands, if they existed in the ancestors of Appendicularians, were almost certainly multi-cellular organs (see account of endostyle, p. 106).

Salensky homologizes these glands with the adhesive suckers of larval Ascidians, which he believes may have been derived from them. Ihle objects that it is not easy to compare two oral glands with three oral suckers, and that they are absent in Oikopleura longicauda and other species believed to be primitive. Although, as we shall see in a moment, if there is any relationship between these structures, the descent must be traced the other way round, it is worth noting that Ihle's first objection at any rate is not fatal to a possible homology, for two reasons. Firstly, in Kowalevskia Lohmann has shown the existence of three ‘ grosse Hautdrüsen ’ in the pharyngeal region, two lateral (?=the oral glands of Oikopleura) and one mid-ventral (1896, Pl. I; cf. my Text-fig. 4, *a*); and secondly, that in Botryllus at any rate the innervation of the larval suckers is by a pair of nerves, one to each lateral sucker, the median sucker merely receiving a twig from the right lateral nerve (Grave and Woodbridge, 1924). Thus the primitive larval condition may have been one with a single pair of suckers, the median one being a later addition ; or if three are primitive, the median one may be represented in Oikopleura by the simpler ‘ Kehldrüse ’ which Lohmann associates with the oral glands, significantly enough, as possibly serving ‘zur Befestigung des Tieres’ inside the ‘Haus’ (1899, p. 380, Taf. II, fig. 15).

But all this is overshadowed by Delsman’s remarkable observations on the development (1910 and 1912), a full account of which is given by Ihle (l. c., pp. 476–7, figs. 27, 28). The oral glands are of endodermal, not ectodermal origin. They are said to be derived from a Y-shaped mass of endoderm, which makes its appearance below the gut, immediately behind the endostyle, which is lodged in the fork of the Y.

Delsman gives his account with some reserve, but believes this mass to originate from remnants of the subchordal gut. The details he gives, however, are of such an extraordinary character that further evidence is necessary before they can be regarded as established. Leaving the question of origin sub judice, there appears to be no uncertainty about the later stages. The Y-shaped mass shrinks and becomes U-shaped (Ihle, fig. 28): this divides into right and left halves, and each half, moving forward, fuses with the ectoderm on each side of the endostyle, and acquires an opening to the exterior.

There is thus a possibility that the Y-shaped mass does not really migrate forwards out of the tail, but arises locally by differentiation from the floor of the pharynx. In that case it would be comparable with the pharyngeal packet of a Fritillaria, but more symmetrically constituted, each half, instead of remaining as an appendage of the pharynx, having severed its connexion with the latter and acquired an outlet to the exterior. The matter, however, must necessarily remain in abeyance until Delsman’s observations have been confirmed by sections or corrected, and the question of a further relic of the Ascidian epicardium hangs on the issue.

But in one respect Delsman’s observations are sufficient even now to bring these oral glands into a highly interesting relation with another problematic group of organs, the cement-glands of larval Teleostomes (Graham Kerr, 1919, pp. 79, 178, &c.), which Graham Kerr has shown also to be of endodermal origin. Reserving, however, my discussion of this resemblance, as well as of the relations of both organs to Ascidian suckers and epicardia (see p. 144), let us turn to Amphioxus, for I must now claim the mysterious ' club-shaped gland ' as the modified vestige of a similar pair of structures.

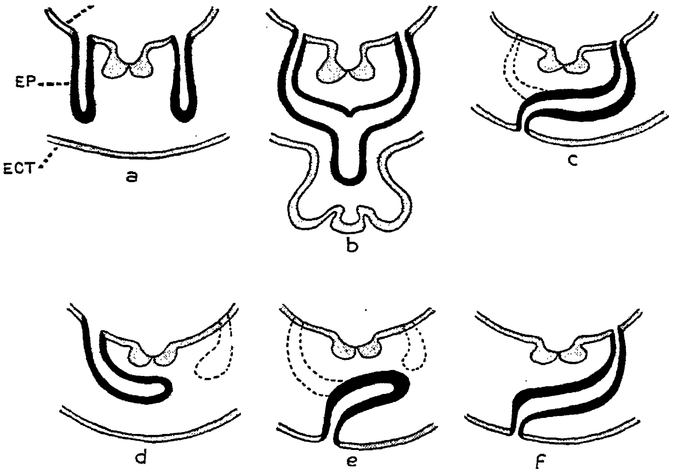
The Club-shaped Gland of Amphioxus, once regarded by Van Beneden and Julin as representing the intestine of Ascidians, and by Willey (after his discovery of its later internal aperture) as the first gill-slit of the right side, undergoes two phases of development. Arising early, just before the mouth and first primary gill-slit, as a shallow groove in the floor of the pharynx, immediately behind the transverse rudiment of the endostyle, it becomes constricted off as a closed vesicle and then opens to the exterior beneath the antero-ventral margin of the left-sided mouth. It retains this structure, from which its name is derived, through the long period with one gill-slit. Although it lies mainly outside the actual right wall of the pharynx, its morphological position of origin, like that of the long left limb of the endostyle in front of it, and of the primary gill-slit behind it, is essentially left-sided.

Then comes the second phase of its development, which provides its blind extremity with a new internal opening into the pharynx, immediately above (i. e. morphologically to the right of) the hinder part of the short right limb of the endostyle (Willey, 1894, figs. 74 and 75). The gland now ceases to be club-shaped and is converted into a transverse tube with an internal orifice on the right of the endostyle and an external orifice on the left of it (Goodrich, 1909, Pl. xv, figs. 32, 33). According to Van Wijhe, the internal orifice, though not formed simultaneously with the gland, may occur as early as the first larval stage with a single gill-slit (1914, p. 69, in confirmation of Legros, 1897).

Willey's interpretation of this puzzling organ as the first gill-slit of the morphological right series was based mainly on the grounds that, without it, the first primary slit (i. e. of the left series) had no antimere, and that the final internal orifice was definitely on the right side; but the objections to this view are surely insuperable. Regarded as a gill-pouch, the bulk of the gland is derived from the left, not the right, side, and the right internal orifice is obviously not the original orifice of evagination. The same objections apply to Van Wijhe's theory of it.

It would be easier to regard the gland as a fused pair of gill-

TEXT-FIG. 11.



Showing the interpretation of the club-shaped gland of the larval Amphioxus as a modified epicardium.

Top row: *a*, epicardia of larval Doliolum or Polyclinid; *b*, epicardium of Clavelina produced into stolon; *c*, club-shaped gland of Amphioxus.

Bottom row: development of the club-shaped gland. *d*, origin of diverticulum outside left wall of endostyle; the right diverticulum arrested; *e*, closure of left orifice of evagination, acquisition of external opening below left-sided mouth; *f*, acquisition of second internal orifice on right side of endostyle (= belated right diverticulum).

ECT, ectoderm; EP, epicardial diverticulum from pharynx, PH.

pouches, of which the right member was retarded in development; but again neither the original nor the final internal orifice falls into sequence with the rudiments of the primary and secondary slits, their close proximity to the endostyle being quite anomalous and at variance with the antagonistic functions normally belonging to gill-slits and endostyle. The absence of an antimere for the first primary gill-slit cannot count for much in the development of Amphioxus, especially as this slit itself is merely transitory.

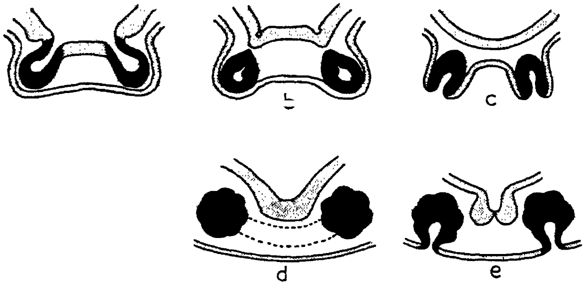
The simplest interpretation is plainly to regard the gland as formed from a pair of pharyngeal diverticula, with separate internal orifices, but fused distally into a single cul-de-sac, which acquires an external opening by fusion with the ectoderm. This Y-shaped tube has then been rendered asymmetrical by a retardation in the formation of its right-sided component, and by early severance of the original left-sided connexion with the pharynx (Text-fig. 11, *d*, *e*, *f*). I need scarcely point out how directly this interpretation points to the origin of the gland as a vestigial epicardium which has undergone a glandular modification and secondarily acquired an external opening. The epicardium of most Synascidians is a Y-shaped tube with a terminal cul-de-sac (Text-fig. 11, *b*), and its original orifices of evagination become closed in later life (Seeliger in Bronn, p. 566). The conditions of asymmetry in Amphioxus account in principle not merely for a retardation in the appearance of the right internal orifice, but also quite naturally for a persistence of this second aperture after the first (left) one has closed. This necessary difference between right and left sides in the time at which the internal orifices open and close has however been secondarily exaggerated by the assumption of a definite glandular function by the organ. I have already cited the case of Euherdmania as an unequivocal example of a glandular modification of the epicardium. The pharyngeal packet of Fritillaria affords another, if more debatable, illustration. In each case the glandular modification appears to have no functional importance but to be merely a step in the retrogressive metamorphosis of the organ. But in the larval Amphioxus the suggestion presents itself that the vestigial epicardium, while undergoing 'glandular degeneration', has been drawn into the service of a larva prematurely hatched and inadequately provided with a normal feeding mechanism. This aspect of the asymmetrical phase of Amphioxus is more fully discussed below (p. 151). In the meantime it can be pointed out that the club-shaped gland is a transitory organ, limited to the asymmetrical larval phase, and with no homologue at all in the larval or adult Balanoglossus, but naturally falling into line with the metendostylic glands in Appendicularians just discussed. The club-shaped gland first directs its products to the outside, like the oral glands of Oikopleura, but when its second internal orifice arises (by simple inheritance) this orifice is retained as a means of diverting the secretion directly into the pharynx. The external orifice has a very significant position in relation to the mouth, and the second internal orifice disappears with the gland itself as soon as the pharynx has become normally constituted and its endostyle fully developed. Thus both the position of the external orifice and the duration of the second internal orifice have probably each been adaptively modified to secure the passage of the secretion into the pharynx of the larva (cf. Van Wijhe, 1914, p. 70).

These simple explanations fit the facts so completely that, after a temporary amazement, I cannot doubt their validity, and must therefore take courage to submit the inevitable corollary. The endodermal ' cement organs ' of larval Teleostomes are also relics of the Ascidian epicardia.

The Cement-glands of Teleostomi.—These ' very interesting and puzzling structures ' (Graham Kerr, l. c., p. 182), which have been variously regarded as modified gill-pouches (like the club-shaped gland), or coelomic cavities (like the Ascidian epicardia), are just paired outgrowths of the pharyngeal endoderm, which become constricted off, fuse with the ectoderm, and then open to the exterior, their glandular surfaces forming the adhesive tips of the suckers (Kerr, l. c., fig. 101). In Polypterus the suckers are situated at the corners of the mouth, but are subsequently carried forward into a preoral position (l. c., fig. 100). It is clearly possible that their original position may have been postoral, a change comparable with that which the premandibular cavities have admittedly undergone. Kerr, indeed, regards them as ' really homologous ' with the postoral cement-organs of Dipnoi and Amphibia, in spite of the difference in their final positions and the apparent absence of endoderm in the organs of the latter groups (l. c.,

p. 181). Like the epicardial outgrowths of Ascidians, the endodermal cement-organs show a marked tendency to fuse into median structures. But for the uncertainty already mentioned as to the origin of the endodermal element in Oikopleura, the cement-glands of Teleostomes and the oral glands of Appendicularians are almost identical (cf. Text-fig. 12).

TEXT-FIG. 12.



Showing the development of Teleostome cement-glands, interpreted as postoral structures, and of the oral glands of Oikopleura (black = endoderm). *a*, *b*, *c*, Teleostome embryos (after Graham Kerr on Polypterus); *d*, embryo of Oikopleura, showing the pair of endoderm masses separating from the earlier common rudiment (after Delsman); *e*, adult Oikopleura, showing secondary fusion with ectoderm.

It may be urged, on the other hand, that if Salensky is right in homologizing the oral glands with the suckers of larval Ascidians, the ectodermal suckers of Ascidian larvae, as well as the oral glands of Oikopleura, may have been derived from the endodermal suckers of Teleostomi, the former by loss of the separate endodermal element (cf. Amphibian suckers), the latter by loss of the suctorial function.

To this method of putting the facts my reply would be threefold: (1) that, in view of the peculiarities common to all the glands under discussion, it is not permissible to detach the club-shaped gland of Amphioxus and the pharyngeal packets of Fritillaria from simultaneous treatment with the oral glands of Oikopleura; (2) that (with reservations already mentioned in the last case) all these glands are readily derivable from epicardial structures, but those of Fritillaria and

Amphioxus are not easily derivable from endodermal suckers; and (3) that, as the Vertebrate pharynx shows vestiges of a former Protochordate structure (endostyle, subnotochordal rod, tongue-bars, see p. 60), it is more consistent to consider the endodermal element in the suckers of Teleostomes as derived from vestigial epicardia.

It should be borne in mind that even if we could be satisfied with an interpretation of the Protochordate glands as modifications of Teleostome suckers, we should merely be substituting one mystery for another, since it is the uncommon complexity of the Teleostome organs which renders them, in Graham Kerr's phrase, so 'interesting and puzzling'. Thousands of animals have invented ectodermal cement-glands, but only Teleostomes have turned endodermal diverticula inside out for this simple purpose. I can well believe with Professor Kerr that there has been an unbroken series of Vertebrate larvae with ventral suckers from primitive Teleostomes to modern Amphibia, but I should express the evolutional change which has occurred along this series rather as an example of the universal tendency to simplification of development. The Teleostomes may have used endodermal glands at the outset, not because endoderm was necessary for suckers, but because glands of that character were already in existence and could be utilized as such from the beginning. Their subsequent simplification affords the best possible evidence that their original complexity was related to some antecedent function which had been superseded.

Although the budding of Ascidians is commonly ignored in discussions of Vertebrate origins, or regarded as a secondary incident of their general degradation, it is difficult to believe, as already remarked, that budding can have arisen de novo at the Chordate level of organization. Assuming then that in this respect Ascidians have retained, and Amphioxus has lost, a primitive Protochordate feature, it is not surprising to find traces of the characteristic Ascidian organ of budding in Amphioxus and in the lower Vertebrates, which must be admitted to have had a Protochordate origin (cf. Van Wijhe, 1914, p. 74).

While the theory of a vestigial epicardium seems to afford a perfectly satisfactory explanation of all these pharyngeal glands, as it does of the reno-pericardial glands of Molgulids (Damas, 1902), it is not irrelevant to add that, so far as I am aware, there is no other structure that can be brought into any conceivable relation with them, unless it be the pair of gastric diverticula in Actinotrocha, which Masterman (1897) interpreted as a pair of lateral notochords. The peculiar position of the droplets secreted by these glands, at the very tips of the cells, and outside the nuclei, makes it highly probable, however, that their secretion is discharged into the blastocele. These glands stand consequently in a class apart, and plainly discharge some important larval function, which it would be interesting to determine more precisely. In view of the observations of Ritter and Davis (1904) on the part played by the gastric epithelium in discharging material into the blastocele of Tornaria, it is possible that the glands of Actinotrocha are also indirectly glaeogenous, both larvae being highly gelatinous, and undergoing the same marked diminution in size at the metamorphosis, when this particular glandular activity ceases. In any case, except for a certain similarity in position, the glands of Actinotrocha seem to possess no features which throw any special light on the origin of the glands we have been discussing.

It should be noted that the reaction of Masterman's 'vacuoles' to osmic acid, in consequence of which De Selys Longchamps interpreted them as fat droplets (1902, p. 581), has been denied in the case of the American species by Brooks and Cowles (1905, p. 89).

I need scarcely add that the presence of epicardial vestiges alone is quite consistent with the previous evidence which points to a Doliolid ancestry of Appendicularians, for although the stolon of Doliolum contains cloacal as well as pharyngeal diverticula, the loss of the cloacal cavity in Appendicularians necessarily carries with it the loss of any trace of cloacal outgrowths.

The case of Amphioxus is more intricate since in the formation of its endostyle and gill-slits it retains features which are more primitive than those in any known Tunicate. The identification of its club-shaped gland as a vestigial epicardium is even embarrassing, since it points to this organ as already an essential feature of the earliest Protochordates, in which case its absence in certain sections of Tunicata must be secondary. This question will therefore require examination, but before proceeding with it, the larval asymmetry of Amphioxus needs somewhat closer study, for not only does it affect the interpretation of the club-shaped gland, but has itself been given a phylogenetic significance which is inconsistent with the views here maintained.

§ 6. The Larval Asymmetry of Amphioxus.

When Amphioxus hatches it does so at an exceptionally early stage, the neural plate being still largely exposed, and only two or three pairs of coelomic segments established. This stage may be regarded as that of a Dipleurula larva, but it has none of the characteristic features of such a larva, being uniformly coated with long flagella and mouthless. It is in fact a mere embryo, prematurely hatched. When the mouth appears, it already exhibits the characteristic asymmetry, as also do the other pharyngeal organs, the right gill-slit of the first pair being absent, the right limb of the endostyle much shorter than the left (or altogether absent ?), and the ‘ epicardium ’ correspondingly limited almost entirely to its left portion, the ‘ club-shaped gland ’. Apart from this asymmetry, however, its grade of organization (except for the absence of atria) now corresponds with that which we might expect in a primitive Ascidian tadpole, viz. mouth, single pair of gill-slits, peripharyngeal band with ventral loop; but, instead of actively swimming, like a tadpole, its physiological condition is as abnormal as its morphological. It possesses numerous coelomic segments but no functional muscles ; still drifts passively, suspended vertically by its general coating of long flagella, and, according to all accounts, continues to do so during the whole of this phase of its larval career (14 days). The significance of these peculiarities seems hitherto to have escaped notice, but their abnormality is quite as great as that of the structural asymmetry, and an explanation which accounts for all of them together is more likely to be correct than any ad hoc speculation which is applicable only to one or two special points.

Dealing with the asymmetry alone, Korschelt and Heider (1893, p. 1461), followed by MacBride (1909), have regarded it as a relic of a hypothetical ‘ pleuronectid ’ ancestry, a view which, apart from other objections, is at variance with the mass of facts, already discussed, which demand a common ancestry for Tunicates and Amphioxus up to a stage of organization well beyond that when the pharyngeal asymmetry is first manifested.

Willey’s suggestion (1891, p. 214 ; 1893, iii ; 1894, p. 159) that it is due to the dislocation of an originally dorsal mouth by the anterior extension of the notochord can hardly be taken seriously, since it rests on the impossible assumption that the dorsal position of the mouth in Ascidian larvae is primitive. This feature, if not a mere anticipation of adult character, admits of a simple explanation in the great development and forward direction of the ventral lobe of fixation, which is obviously a functional adaptation.

The ‘ Tremostome ’ theory of Van Wijhe solves the problem of asymmetry in accordance with some remarkable facts, but leaves a more difficult problem in its place, viz. why the original mouth should have closed. The only suggestion offered by Van Wijhe is that, in connexion with the rotation of the flagellated embryo observed by Hatschek, ‘ the first gill-slit of the left side occupied the most favourable position for an ingestive aperture ’ (1907, p. 70). Apart from the fact that Hatschek’s description of the rotation ‘ von rechts nach links ’ is ambiguous, and that both adult Amphioxus (Franz, 1924) and larval Ascidians (Grave, 1920) rotate in a direction opposite to that which Van Wijhe appears to have assumed (viz. in these forms, clockwise as viewed from behind, or from left to right as seen from above), the ‘ advantage ’ of a left-sided mouth is surely dubious. The larva is advancing all the time that it is rotating, so that a mouth in front would get as much, if not a greater, advantage from the spiral rotation as would an opening on the side. As a matter of fact a ciliary feeding current is capable of maintenance just as well, and usually much better, in the absence of any locomotive movements at all. A rotating scoop, if efficient, would flood the pharynx with more water than it could filter; but there is no evidence that the larval mouth is fitted to act in this way.

The explanation now offered is based on the relative yolklessness of the Amphioxus egg. In general it is not impossible that a yolkless egg may be primitive; but in the groups under immediate consideration it is a complete anomaly, and falls into the same category as the small pelagic eggs of Teleosts, the little-yolked eggs of Passerine Birds, and those of Mammals. If, therefore, we assume that the yolklessness of Amphioxus eggs is a secondary feature—a change from a more highly yolked condition in the Protochordate ancestor—a chain of consequences is set up which accounts both for the muscular feebleness of the larva and its structural asymmetry. For though the thin and diffuse distribution of yolk throughout the egg, and then throughout the germ-layers, ensures a rapid and even differentiation of the latter up to a certain point, the slightness in its amount limits the process, and the embryo hatches long before it is equipped with the normal functional mechanism for feeding and swimming. Like a Passerine chick, or a Marsupial babe, it is precocious in form, but retarded in function, and, as with them, its defective condition sets up an immediate demand, at the expense of everything else, for a provisional feeding mechanism. In the parallel cases mentioned, the demand is met by parental attentions and maternal modification; but here the larva is dependent on itself, and, metaphorically speaking, must improvise something out of the rudiments of an apparatus functionally adapted to a different set of conditions. At any rate a study of the asymmetrical pharynx reveals the possibility of an explanation of it on these lines.

Exactly how this abnormal pharynx works we do not know from observation and experiment, but certain peculiarities of structure indicate its main principles. The mouth, unlike the mouth of the adult, or of Tunicates at any stage, is powerfully and peculiarly ciliated (Willey, 1891, p. 211), the gill-slits apparently less so, at least in the earlier stages (cf. Goodrich, 1909, Pl. xv, figs. 32, 36). This means that the current is produced by the mouth, and not, as is usually the case, by the cilia of the gill-slits. If a strong stream were to be drawn through the first and each successive gill-slit, food particles and water together would sweep almost uselessly through the pharynx, since the compression of the body is so extreme that there is no room for a filter of mucilage between mouth and gill-slits, as in Appendicularians, and there is no long endostyle, as in the adult, to coat the walls themselves with slime. From the arrangement of parts it is probable that the long, deep-seated, oral cilia (Willey, l. c.) beat the water in a thin diffuse stream against the inner left wall of the pharynx upwards, downwards, and sideways, but especially downwards (where food particles are specially noticeable in preserved specimens), so that food particles can be collected by the peripharyngeal bands which encircle the mouth at a slight distance within the pharyngeal cavity.

If the current were to set strongly in against the right wall of the pharynx, it is difficult to understand how any food particles could be kept from dropping at once into the exhalant streams converging towards the gill-slits. But if the mechanism works in the way suggested, and indeed in whatever way it works, so long as the oral cilia are the chief current-producers, the larger and more numerous the outlets, the gentler and more diffuse will be the streams; so that the dilatation and weak ciliation of the gill-slits and their multiplication posteriorly are exactly the adaptations required for a successful expedient in the absence of a filter across the intake. Presumably the endostyle at this stage works as in Appendicularians by directing a flow of mucilage along the peripharyngeal bands, especially the lower (left) one, which, like the limb of the endostyle continuous with it, is better developed than the upper (right) one, and lies like a trap along the narrow floor of the pharynx between mouth and gill-slits in these early stages (Goodrich, l. c., fig. 33). Later on, when the endostyle has grown backwards from the apex of its convergent limbs, and the median flagella have been developed (see p. 90), the dominant direction of its ciliary activity presumably changes from longitudinal to lateral, as in the adult.

More than this we can scarcely infer, except, as already suggested, that the club-shaped gland almost certainly plays an important part in supplementing the function of the imperfectly developed endostyle, as Goldschmidt (1905) and Van Wijhe (1914) have previously maintained. We can point out that the gland produces and contains material which is probably utilizable, and that, when the larva is vertically suspended and drifting slowly with the surrounding water, the position of its external orifice near the anterior end of the lower lip is ideally situated for supplying the edge of the mouth with mucilage, and indirectly—through the action of the oral cilia—the important prebranchial zone between mouth and peripharyngeal bands which cannot be served by mucus from the endostyle. The special anterior tufts of cilia described by Willey (l. c., pp. 211–12, figs. 20, 21, 23), together with the sub-oral tract of small cilia, may play an important part in sweeping the secretion into the mouth. The subsequent internal aperture, which is peculiarly long and slit-like, must first supplement, then replace, the external pore in function, until the asymmetry is in course of rectification, but by that time the structural conditions are too complex for any safe opinion on the actual direction of the currents or the part played by secretion from the gland.

If this is a correct exposition of the larval feeding mechanism, it is no more necessary to force a phylogenetic meaning into it than in the corresponding cases of the chicks of Sparrows and the embryonic babes of Marsupials, and even the most ardent Haeckelian has not yet ‘ explained ’ their abnormalities in terms of naked, blind, and suctorial ancestors.

A certain asymmetry, or left-sidedness, is noticeable both in Rhabdopleura (Schepotieff, 1904) and the Tunicata, so that a slight tendency in this direction may be an inheritance from prechordate ancestry; but its extreme development in Amphioxus larvae I regard as a larval adaptation, consequent on the secondary yolklessness of the egg. The mouth is enlarged to colossal proportions and specially ciliated to collect food. It cannot be confined to its normal place, so it invades the side to which there is perhaps an inherited tendency, and displaces to the right side the gill-slit that belongs to the left, all the normal right-sided organs being temporarily suppressed in development. As the mouth enlarges the gill-slits, weakly ciliated (see Goodrich, 1909, Pl. xv), multiply and dilate, thus avoiding any intensification of the oral stream by the formation of ‘ flues ’. Without these compensatory adjustments in the early stages, feeding would be impossible, or at least involve great waste of food; but, with these arrangements, and by postponement of all muscular activity and unnecessary organ-formation, such as atrial folds, the larva grows in bulk, the mouth turns round into its proper place, the gill-slits follow suit, the two limbs of the endostyle unite and grow backwards, supernumerary gill-slits of the precociously developed left series are absorbed, and epibranchial flaps eventually grow downward over the gill-slits of both sides to form the belated atrium. Adaptation and self-regulation could hardly go farther, but there is nothing in the whole chain of events which is not sequential on the original disturbing factor, the secondary reduction of yolk in the egg.

Nevertheless, although the ultimate product of this extraordinary development is a bilaterally symmetrical creature which is universally accepted as the simplest type of Vertebrate normality, the morphologist, who believes in the possibility of reconstructing phylogeny, may well ask the question: Has all this chain of larval modifications left no effect on the adult form and structure? Is the final product identical in essential features with the adult form of that earlier life-history which began with a more normal provision of yolk in the egg?

The analogies which I have given of yolk-reduction do not help us here, for the Sparrow and the Marsupial are carried over the difficult post-embryonic period not by conspicuous modifications of their own structure, but by the attentions and modification of their parents. But in Amphioxus the structure of organs, and their relations to one another, are definitely changed in the larva, and these changes are inherited as truly as any adult changes may be inherited. The mouth develops from the outset on the left side, and the left gill-slit on the right side, though the larva is freely and vertically suspended. The subsequent rectification of these relations masks, but does not explain, the morphogenetic problem involved. If Amphioxus could reproduce before the rectification took place, a new type of organism would have come into existence, the distinctive features of which were consequential on the primary mutation of yolk-reduction. Indeed, if Goldschmidt (1905) is right, that Amphioxides is capable of reproduction, this has already happened (see, however, Gibson, 1910, p. 239).

It is therefore possible that certain distinctive features of the adult Amphioxus may be primarily due to the provisional larval adaptations we have described, and that they have been retained, either because rectification was physically impossible, or because, in association with changed habits of life, they yielded a new viable combination which needed no rectification. One such feature, indeed, has long been recognized as a result of Van Wijhe's observations (1889), viz. the innervation of mouth and buccal (pre-oral) cavity exclusively by nerves of the left side. To this two points in particular may be added: the form of the atrium, and the multiplication of gill-slits. We have already given reasons for the opinion that the formation of the atrium is secondarily retarded, and that the multiplication of gill-slits forms a vital part of the provisional larval feeding mechanism, so essential that a number of the first-formed gill-slits are subsequently absorbed, when the right series begins to appear. A bionomical condition of both these features is undoubtedly the peculiarly passive drifting life of the larva, for no larva could safely combine such extensive fenestration of its body-wall with active habits of muscular locomotion.

It follows that the unusual multiplication of gill-slits in Amphioxus, i.e. its elongated pharynx, and the single ventral atrium, may be secondary consequences of the larval modifications induced by the diminution of yolk. And when to this possibility we add the evidence derivable from the history of the club-shaped gland, we submit that in all probability the ancestors of Amphioxus were essentially primitive sessile Ascidians, with an active muscular larva of a generalized Ascidian type; and that Amphioxus owes much of its actual organization to secondary modifications consequent on the elimination of fixation, metamorphosis, and budding from its life-history, and on the reduction of yolk in its egg.

In its possession of protonephridia, however, Amphioxus displays a primitive feature unique among Chordata, but the analogy of Actinotrocha renders it probable that we should regard this as the retention of a larval character, not without possible relation to the absence of genital ducts. We know that in Teleostei the secondary reduction of yolk and the introduction of pelagic spawning have been followed by diminution and eventual atrophy of the oviducts, and the reduction of genital ducts in Appendicularians is another comparable illustration. Although Amphioxus is not exactly a pelagic animal, its organization for burrowing is limited to the adaptation or extension of certain features previously in existence (notochord, atrium, incipient metamerism, general shape), and some of those pre-existent features (neuro-muscular metamerism, notochord) point emphatically to an active larval life in the past before the period when yolk-reduction had set in. Another larval character of the adult Amphioxus is the absence of a pericardium, which is a constant feature in adult Tunicata, even in the degenerate Appendicularians.

On all points we reach the conclusion that the organization of Amphioxus is essentially a paedomorphic transformation of a sessile Protascidian type, due primarily to a prolongation of the originally active larval life, with the elimination of fixation and metamorphosis, and modified by direct and indirect consequences of a reduction of yolk in the egg, and by minor adaptations to a sand-burrowing existence.

This view of the relationship between Amphioxus and the Tunicata finds a somewhat striking confirmation in a comparison of the caudal regions. Hitherto the Ascidian tail has been generally regarded as equivalent to the post-anal region of Amphioxus, specialized for larval life by degeneration of the myotomes. As a matter of fact the young Amphioxus larva possesses a highly characteristic caudal fin long before the segmented ‘ tail ’ of the adult begins to develop (Korschelt and Heider, figs. 875–6); and its peculiar striations, which simulate fin-rays, are strikingly similar to those in the cuticular tail-fin of larval Ascidians, and to those of the youngest larval Teleosts, as already remarked upon by Lankester and Willey (1890, p. 457). On the histological nature of these resemblances, which may well be clinching, I hope to report later. In the meantime it should be noted that at the metamorphosis, when the formation of myotomes and tail is completed, this larval fin is pushed off and replaced by the adult fin, which possesses a radically different structure (Lankester and Willey, l.c., figs. 1, 4; Korschelt and Heider, figs. 877–8). Now, although histological details are lacking, it is remarkable that in Asymmetron the larval fin is borne almost entirely on an unsegmented caudal process which contains extensions of notochord and nerve-cord, but no myotomes. At the metamorphosis this fin appears to migrate forwards (Andrews, figs. 5, 3, 4), but is more probably replaced by a new one in the segmented region, while the appendage persists in the adult as a finless ‘ urostyle ’. This appendage, and the larval fin of Amphioxus, must be homologous with the Ascidian larval tail.

Thus two phyletic stages are represented in the ontogeny of the Cephalochordate tail—a primary stage, comparable with that of Ascidian larvae, and a subsequent definitive stage associated with the secondary metamerism of the body. In Asymmetron these two stages are distinct and successive; in Amphioxus they are blurred by suppression of the primary caudal appendage altogether. In other words the Ascidian tadpole is not a reduced Amphioxus, but Amphioxus (with certain obvious reservations as regards the coelom) is an Ascidian tadpole which has lost its tail and undergone a secondary elongation and segmentation of its body. The post-anal region of Amphioxus, with its myotomes and caudal fin, is a new and later differentiation, unrepresented in Ascidians—an adaptation of its body to the retention of a free life beyond the originally restricted larval stage.

§ 7. BUDDING AND THE ORIGIN OF EPICARDIA.

The original nature of the pharyngeal diverticula which fuse to produce the stolonial septum of the Clavelinid Synascidians is still unsettled, and there are legitimate differences of opinion whether these structures arose as such in connexion with the budding process, or became secondarily involved in the process after previously discharging a purely somatic function (cf. Seeliger in Bronn, pp. 564, 921). The situation at the present time may be defined, I think, as follows.

It is fairly clear that the occasional relations of the epicardia to the heart (e.g. in Clavelina), to which the pharyngeal diverticula owe their name (Van Beneden and Julin, 1887), are secondary. The recurrence of these relations in the reno-pericardial organ of Molgulids is all the more noteworthy (Damas, 1902). In Ciona the pericardial rudiments have long been known to arise independently (Willey, 1893, i; Damas, 1899; De Selys Longchamps, 1901); and both in Clavelina and Distaplia Julin himself finally asserted the complete developmental independence of the two organs (1904, p. 545). The epicardial diverticula, when present, always arise later than the pericardial. In other words Van Beneden and Julin's conception of 'procardial' outgrowths, which subsequently become separated into epicardial and pericardial elements, has gone, together with the theoretical superstructure based upon it. The interpretation of the epicardia as modified coelomic diverticula, possibly 'collar cavities' (Seeliger in Bronn, p. 925; MacBride, 1914), loses an important argument in its favour by the withdrawal of all evidence of a developmental connexion with the pericardium (cf. Sedgwick, p. 16).

Similarly the expansion of the epicardia in Ciona to form a pair of perivisceral cavities seems to have no greater significance than the similar expansion of peribranchial diverticula in Botryllus and the Polystyelidae or Amphioxus itself. The fact that ectodermal diverticula can discharge a pseudo-coelomic function very similar to that of the epicardial sacs in Ciona seems to show that in both cases the 'perivisceral' function is a secondary one, and lends no support to a theory of their coelomic origin.

Finally, the fact that the epicardial diverticula by distal fusion (Clavelina, Polyclinids, &c.) or by prolongation of the left one alone (Ciona) undoubtedly function as a septum between afferent and efferent blood-channels, either in the abdomen alone or in stolonial or pseudo-stolonial extensions, is paralleled by a similar modification of peribranchial extensions in Archiascidia according to Julin (1904), in Polystyelids (De Selys Longchamps, 1917; Oka, 1926), and possibly in Perophora (Lefevre, 1898), though the last case rests on indirect evidence and is at variance with observations by Kowalevsky (see De Selys, 1901, p. 514) which have not yet been disproved.

This allelomorphism of endodermal diverticula of the pharynx and ectodermal diverticula of the atrium subservient to the same physiological functions is also recognizable in the types of budding among Ascidians, which with a few insignificant modifications fall into one or other of two classes according to the origin of the inner layer of the triploblastic bud rudiment. These types can be distinguished as pharyngeal and peribranchial respectively and are characteristic of different natural groups of Ascidians. In the Clavelina-Distomid-Polyclinid series the inner vesicle of the bud-rudiment is derived from diverticula of the pharynx (i. e. endoderm), but in the Botryllid-Polystyelid series from diverticula of the peribranchial epithelium (i. e. ectoderm). It is not known whether Julin's Archiascidia reproduces by some form of budding or not, but if it does—and this is highly probable—the inner vesicle is almost certainly derived from the abdominal septum, as in Polyclinids; but this septum, as already remarked, is formed in Archiascidia from peribranchial, not pharyngeal, diverticula if Julin's remarkable account is to be accepted. It is greatly to be regretted that Julin gave no illustrations to support his account of the development of the septum in this rare and remarkable animal, especially as the sections he figures of the adult structure point on the face of things to a simple origin of the septum from posterior diverticula of the pharynx as in Clavelina (cf. his figs. 8 and 9), and the peculiar postero-dorsal series of stigmata (figs. 1, 2) require an extension of the peribranchial cavity on each side exactly corresponding with the extensions which, according to his account, atrophy and disappear after giving rise to the abdominal septum.

However, apart from the doubtful cases of Archiascidia and Perophora, the contrast between the two types of budding is clear and distinct, and corresponds with well-established systematic differences between the two series of Ascidians based on other characters. The cleavage between the two series on grounds both of adult structure, larval characters, and mode of budding is indeed so sharp and clear that the group of Ascidiacea (i. e. the fixed Ascidians) must be regarded as consisting of two perfectly distinct stocks, related to one another only in the sense that both are derivable from some third stock which is still unknown, and possibly quite extinct.

These two stocks, which have not hitherto been formally recognized, I propose to term Endoblastica and Periblastica respectively. The recognized groups fall into them as follows:

A. Endoblastica.—Pharynx produced behind the endostyle into a pair of diverticula ('epicardia') which separately or by distal fusion form a septum (which may be distinguished as the 'endophragm') between afferent and efferent blood-channels in the abdominal region or in the branches of a single ectodermal outgrowth of mid-ventral origin (stolon and test-vessels); endophragm absent in solitary forms only and then replaced by folds of the walls. Larva with cerebral eye and otolith, and a ventral adhesive organ with three suckers, but no peripheral processes (except Didemnidae). Peribranchial cavities developed before the cloaca. Brain with subneural gland. Gonads unpaired. Buds, if produced, deriving their inner vesicle by outgrowth from, or by fragmentation of the endophragm or epicardia (with rare modifications).

1. Aplousobranchia,[1] Lahille, 1890 (but more correctly Haplobranchia), amended by Garstang (1895) by elimination of Doliolidae and Pyrosomidae = Krikobranchia, Seeliger, 1907.
2. Phlebobranchia, Lahille, 1890 (and Garstang, 1895) =Dictyobranchia, Seeliger, 1907.

B. Periblastica.—Pharynx without epicardia or endophragm, or at most with modified vestiges (Molgulids). Larva with one cerebral sense-organ only, with or without ventral suckers, but with a ring of ectodermal papillae around the adhesive area. Peribranchial cavities formed as outgrowths from cloaca. Brain with supraneural gland (rarely lateral or subneural). Gonads usually paired. Buds, if produced, deriving their inner vesicle by outgrowth from the outer peribranchial epithelium or from a pair of posterior peribranchial diverticula. Centrifugal test-vessels supplemented or replaced by peripheral, all without a septum (constricted or ' doubled ' in Molgulids).

1. Stolidobranchia, Lahille, 1890 (and Garstang, 1895) =Ptychobranchia, Seeliger, 1907.

(?) 2. Hemibranchia, nom. nov. for Julin's Archiascidia if its abdominal diaphragm should prove to be of peribranchial origin, as alleged (i. e. an ' ectophragm ').

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[1] I have given these names and synonyms in systematic detail with their respective dates of publication in order to take this opportunity of remarking upon Seeliger's procedure in assigning new names to groups already established in 1895 under names proposed by Lahille (1890), who, in spite of some mistakes, is undoubtedly entitled to the credit of having laid the foundations of the modern classification of Ascidians. Seeliger's action is the more indefensible as he notes with approval the changes I made in Lahille's scheme (Bronn, p. 1061) and adopts them without modification. In spite of Hartmeyer's retention of Seeliger's terms, these, if only on grounds of priority, must clearly give way to Lahille's, either in their original form or as amended by me. The correction of mere spelling embodied in the substitution of ' Haplobranchia ' for the barbaric ' Aplousobranchia ' may reasonably be adopted, without any injury to Lahille or breach of sound principle.

The inclusion of Archiascidia, however, would involve omission of the special larval peculiarities of the Periblastica, since its own larva in those respects is typically Clavelinid.

[3. Aspiraculata, Seeliger, 1907. This suborder, which was proposed for the single species Hexacrobylus psammatodes of Sluiter, falls in here; but as this type is admittedly of Molgulid extraction, and closely related to Bourne's Oligotrema, the group should properly be suppressed and replaced by a subfamily of the Molgulidae, e.g. Oligotrematinae, to include Oligotrema and Hexacrobylus.]

It will be noticed that the first group (A) includes both long-bodied and short-bodied forms, and that the second group (B), again with the uncertain exception of Archiascidia, includes only short-bodied forms. Sluiter's attempt to found a primary subdivision on this basis (as Merosomata and Holosomata) broke down owing to the cross-relations of such forms as Ciona, Rhopalaea, Ecteinascidia, and Diazona. As now arranged the cleavage between the two primary groups is sharp and absolute, with regard both to somatic, larval, and blastogenetic characters, although, as in other phyletic classifications, it is impossible to draw a hard and fast line between these two series on the basis of any one selected character. In group A the test-vessels are septate and radiate from a central point (i.e. centrifugal), which coincides with the base of the stolon when this is present; in group B the centrifugal system is supplemented or replaced by test-vessels having a diffuse or peripheral origin, septa are absent, and budding, when it occurs, is parietal instead of stolonial, although without any direct relations to the peripheral ring of test-vessels. The gonads are unpaired in (A), usually paired and parietal in (B).[1]

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[1] For systematic purposes terms based on this contrast would be more convenient than the descriptive terms I have used in the text, since every animal develops gonads, and budding is more or less sporadic. Perrier's (1897) term Pleurogona (i.e. gonads parietal, usually paired) is the exact equivalent of my Periblastica; and if we reunite his ill-separated

Clearly in this contrast we have come upon a crucial point in Ascidian evolution, and it is important to find an explanation. The clue once more is provided by Doliolum, or rather by the details of the budding process in the Thaliacea generally, for in these forms (with the possible exception of Anchinia) there is neither epicardial budding as in the Endoblastica, nor peribranchial budding as in the Periblastica, but transverse division of a median stolon which contains both pharyngeal and cloacal diverticula. To Julin (1904, p. 547) belongs the credit of first pointing out the significance of these relations: 'les deux types connus du bourgeonnement des Ascidies doivent dériver d'une forme primitive, réalisée chez Doliolum'; but the paper in which he promised to develop his ideas seems never to have been published.

In the meantime De Selys Longchamps (1916 and 1917), by his work on the Polystyelids, has not only widened the basis of comparison, but has reached the same conclusion as Julin, with a slight, but important, qualification on the phylogenetic question: 'Il faut bien admettre que les Thaliacés, encore que secondaires au point de vue de la structure des adultes et de leur embryologie, sont primitifs en ce qui concerne la blastogénèse (formation du stolon) . . . et . . . que la forme ancestrale commune aux Thaliacés et Ascidiacés était douée d'un mode blastogénique pharyngopéribranchial, différant en réalité fort peu d'une simple division transversale, avec laquelle la blastogénèse des Thaliacés a conservé tant d'analogie' (1917, p. 271). De Selys, however, refrains from any development of these phylogenetic considerations, and raises them merely to emphasize his view of the secondary nature of the 'stolonial' (=epicardial) mode of budding in Tunicata, as against the primitive role which Korschelt and Heider were inclined to ascribe to it (l. c., pp. 269, 272).

There the matter rests; and the question is now whether

'Hemigona' and 'Hypogona' under his own alternative term Enterogona, but with a widened significance (i.e. gonads unpaired, lodged in the intestinal loop or in an abdominal extension of the body), we obtain the systematic equivalent of my Endoblastica.

this new orientation towards the facts helps us to comprehend the evolution of budding in Tunicata and thereby, in conjunction with other considerations, form some idea of the form and habits of the primitive Tunicate from which both Ascidiacea and Thaliacea have been derived. The evidence derived from the modes of budding, which seems to make the Thaliacea more primitive than the Ascidiacea, has to be reconciled with the unequivocal evidence already given that the Thaliacea have been derived from fixed ancestors (position of cloaca, structure and functioning of gill-slits and endostyle, degeneration of oozooid).

When the modes of budding in Tunicata are traced back to a process which ' differs in reality very little from one of simple transverse division ', it is nevertheless to be noted that the process in question differs materially from true fission as exemplified, for example, in Phoronis (Harmer, 1917), in the fact that the functional somatic organs are not themselves divided. In Phoronis both limbs of the intestinal loop are cut through in the process, and there is regeneration on each side of the dividing plane. In Thaliacea the body is not divided, but only a process of the body which contains special prolongations of the organs immediately adjacent to it. It is as if we had before us, not an adult Phoronis, but an Actinotrocha in which the ventral invagination had been everted, and then invaded by special prolongations of the descending and ascending portions of the gut instead of by the gut itself. This would constitute the equivalent of the Thaliacean stolon, and in the transverse division of such a stolon we should have a real parallel to the mode of budding now regarded as primitive in Tunicata.

This comparison will serve, I think, to show that the interpretation of Tunicate budding as in any sense related to transverse fission is superficial and incorrect, the segmentation of the stolon being a secondary feature having no connexion with such a process. Korschelt and Heider (1893, p. 1365) long ago pointed out that the axial relations of the first four ascidiozooids to the stolon in Pyrosoma are completely at variance with any theory of transverse (somatic) fission, but easily interpreted in terms of stolonial budding.

The difference between the typical Thaliacean stolon and that of Ascidians in the order of development of the buds is also readily correlated with a change from sedentary to pelagic conditions. The stolon of a Perophora or a Clavelina provides anchorage for the colony as well as nutrition for the buds, and distal growth is a necessity of the situation. But the stolon of a pelagic Tunicate is free from this restriction, and the establishment of a proximal zone of growth follows inevitably from the conversion of an undivided, leisurely branching, stolon into an unbranched organ devoted to the rapid production of free buds.

Thus, apart from differences in its internal constitution, the monaxial or trailing stolon of pelagic Tunicates can be consistently regarded as an adaptive modification of the creeping stolon, and some confirmation of this view is provided by Anchinia. Although data are still lacking for a final conclusion on certain points, we know that in Anchinia a slender stolon-like thread is present along the dorsal side of the colonial stalk to which the free buds are found to be attached (Barrois, 1885, p. 203, Pl. viii, figs. 1, 3). The stalk itself almost certainly corresponds to the dorsal stalk of Doliolum, which is represented in the gonozooids of Anchinia by a long tentacle-like process from the dorsal edge of the cloacal aperture. In our ignorance we can only suppose that oozooid and gonozooid are more or less alike, and that the ventral stolon in the oozooid bends up one side of the body and grows dorsally and backwards along the cloacal tentacle, which becomes the colonial stalk. Something similar to this creeping growth of the stolon over the parental body takes place, as is well known, in Salpa (Korschelt and Heider, 1893, p. 1399, fig. 849), but there the stolon glides along a track carved out for it in the test substance, and then trails freely behind, and the process is easily reconciled with proliferation from the base. It is probable, therefore, that the stolon of Anchinia, by creeping over the parental body, is repeating, in a very novel way, the original mode of growth which characterized its fixed ancestors, for presumably the whole of the stolon rests on a substratum provided by the parental body. In this mode of growth we have the probable explanation, as Barrois suggests, of the remarkable migration of the free pro-buds of Doliolum, which repeat the process; but, although these pro-buds are produced by fragmentation from the free-end of the stolon, which must therefore grow from its base, it by no means follows that this rule also obtains in Anchinia, in which the slenderness of the stolon, in relation to the length of its journey, seems to require retention of a terminal growing-point as in Clavelina—unless the whole stolon is literally dragged along by amoeboid phorocytes, as the individual buds are transported in Doliolum (cf. Korotneff, 1884; Korschelt and Heider, 1910, fig. 610).

These various considerations all point to one and the same conclusion, that the ancestors of Thaliacea were sedentary forms which reproduced asexually by budding from a ventral stolon. The differences between Pyrosomata and Myosomata (Doliolidae and Salpidae) in regard to the form of the pelagic colony are considerable at first sight, and might seem to justify a case for the independent origin of these two tribes from the ancestral sedentary stock, but the cross-relationships are too intimate to render such a view tenable. If the ancestor of Pyrosoma was a colonial Tunicate having the form of a Cyathocormus (Oka, 1913), the primary stolon must have had the form of a wreath of buds around the larval body (cf. Diplosoma, Lahille, 1890, fig. 64), and such a wreath, by further elongation and more extensive proliferation, may well have given rise to the winding stolon of an Anchinia or a Salp. Similarly if the stolon of Doliolids was directly preceded by a creeping stolon in an ancestor of the Social Ascidian type, this ancestor was nevertheless as closely related to the other as a Clavelina is to a Distaplia. An independent origin of the two sections from genera more remote from one another than those is precluded by their possession of too many peculiar points of resemblance.

But apart from the constitution of the stolon, there are several obstacles to the view that the Thaliacea are derivable from the Clavelinid group of Ascidians (Haplobranchia). As pointed out by me in 1895, the structure of the internal armature of the Pyrosoma pharynx is quite definitely Botryllid, not Clavelinid, in character; and in the present paper I have drawn attention to the peculiar way in which the gill-slits of Doliolum apparently develop as pairs of protostigmata (p. 113), a method which is as remote as possible from the Haplobranchiate type, but closely approaches that of the Stolidobranchia, though still more primitive. Equally significant are the special resemblances between Salpa, Doliolum, and the Stolidobranchia as regards the mode of development of the cloaca, to which my daughter and I have drawn attention in our account of the development of Botrylloides.

In these facts, then, we have good grounds for the belief that the Thaliacea are the pelagic representatives of an early stock of sessile Tunicata, antecedent to all known types of Ascidians, and in which the ventral stolon of the Haplobranchs was combined with the pharynx of a primitive Botryllid. To these features we must add that in all probability the constitution of the stolon was already Thaliacean in the sense that it contained both pharyngeal and peribranchial diverticula. The origin of this latter complexity remains for consideration.

As budding from a ventral stolon characterizes both the Thaliacea and the Endoblastic Ascidians, it is reasonable to assume that it characterized the earliest Tunicates, provided the absence of such a stolon in the Periblastica can be interpreted as an example of secondary loss (see p. 173). If this be admitted, a connexion with Pterobranchia is at once afforded by the correspondence in position of the ventral stolo prolifer in both groups. Except for the presence or absence of an endodermal septum, there is complete agreement between Rhabdopleura and Clavelina in their methods of asexual reproduction: both have ventral stolons from which the summer colonies arise, and both form resting ('winter') buds by a distinct process of stolonial fragmentation (Schepotieff, 1904; Kerb, 1908). Moreover, in view of the relations now established between the coenoecia of modern Pterobranchia and those of the ancient Graptolites (Schepotieff, 1909), the wide variation of the latter, both in structure and in conditions of fixation and flotation, suggests many possibilities affecting the phylogenetic outlook. A drifting sicula with its Monograptid or Digraptid chain of buds must have been something remarkably similar to a Salpa-nurse with its trailing stolon. The shape of the sicula is often different from that of the rhabdothecae. 'The sicula itself ceases to grow, as a rule, after the first thecae are budded, and sometimes it becomes obsolete or absorbed' (Zittel in Eastman, 1913). Clearly Graptolites showed all the phenomena we discuss in pelagic Tunicates—differentiation between oozooid and blastozooid, arrested development of oozooid, precocious budding; and it should be remembered that neither in Cephalodiscus nor in Rhabdopleura can we yet assert that the larva completes its development after fixation into a 'normal' adult individual resembling the blastozooids.

In view of these many resemblances, there is clearly a possibility that budding from a ventral stalk of fixation is a feature which Tunicata have inherited from Pterobranch ancestors, and we can test this hypothesis in the usual way, by assuming it and seeing whether it works. The first result of assuming it is that we obtain a series of stages in the varying constitution of the stolon as follows:

(1) The stolon encloses a pair of coelomic diverticula alone (Pterobranchs).

(2) The stolon encloses mesoderm together with paired pharyngeal and cloacal diverticula (Thaliacea).

(3) The epithelial diverticula are reduced either to pharyngeal outgrowths alone (Endoblastica) or to peribranchial outgrowths alone (Periblastica), but in the latter case the stolon as a single median outgrowth has disappeared, or undergone disintegration, a phenomenon not without parallel in the Endoblastica (e.g. Distaplia, Didemnidae).

We have to bear in mind the uncertain nature of the stolon of Anchinia (Thaliacea), which according to Barrois (l. c.)

contains only an axial core of loose cells which he regards as endodermal. These may possibly represent an epicardium, but more probably result from the breakdown and fusion of both pharyngeal and peribranchial elements. If the latter view should be confirmed, and for Dolchinia also (Korotneff, 1891), the Thaliacean stolon, in contrast to that of Ascidiacea, would be consistently ' amphiblastic '.

In the stolon of Pterobranchs there appears to be no special endodermal diverticulum from the parental body. The existence in Rhabdopleura of a central endodermal core between the coelomic diverticula was maintained by Fowler (1905) but denied by Schepotieff (1904, 1907), who derives the endoderm of the bud from wandering cells which collect together on either side of the median septum (i.e. mesoderm). In Cephalodiscus the endoderm of the bud is definitely produced by a special invagination of the ectoderm as in larval development (Masterman, 1898). This layer in Pterobranchs, behind the proboscis region at any rate, is relatively unspecialized.

In Tunicates, on the other hand, the ectoderm of the body-wall is everywhere specialized for the production of test material, and is thus rendered incapable of playing a formative part (Hjort, 1896). We have thus to account for the entrance into the stolon of special pharyngeal and cloacal derivatives which could take over the development of the internal organs. It seems natural to connect this problem with the phenomena of periodic degeneration and recrescence which are widespread among small plankton-feeding organisms, and well known in Ascidians themselves (see especially Caullery, 1895). The whole body may degenerate and leave only a dormant residue of undifferentiated material; or, as in Diazona (Della Valle, 1884; Korschelt and Heider, 1893, p. 1360), the thoracic region only may atrophy, leaving the intestinal loop and heart intact (cf. Aphanibranchion, Oka, 1906, and the remarks of Seeliger and Hartmeyer on it in Bronn, pp. 1229, fig. 229). Caullery has described very similar phenomena in Didemnidae, degradation of the thorax, followed by regeneration, being a constant feature of Diplosoma gelatinosum, while the abdomen remains usually unaffected (l. c., pp. 18, 59, 60). It is in this latter type of thoracic atrophy and regeneration that I think we find the key to our problem. Caullery has shown the intimate relationships between budding and regeneration in Ascidians, and that the epicardium is as much an organ of partial regeneration as it is of complete budding both in Polyclinidae, by experiment (l. c., p. 118), and in Didemnidae naturally (p. 133). In these cases, however, the epicardium is already established as 'le tissu régénérateur', and I wish to consider the facts in relation to an antecedent condition, before the epicardium had become an organ capable of regenerating everything. It is likely that its powers of regeneration were more restricted at the outset.

These remarks with regard to the epicardium are equally applicable to the peribranchial epithelium in the contrasted group (Periblastica). In Polycarpa, at any rate, it is known that after spontaneous evisceration, which occurs in various genera, the whole alimentary canal is regenerated by folds of the peribranchial wall (De Selys Longchamps, 1915). It is obvious that the history of regeneration in Tunicates has run a course parallel to that taken by budding, so that in the end we are confronted with two alternative modes of wellnigh total regeneration, viz. in Endoblastica from the epicardium, in Periblastica from the atrium. Clearly the common ancestor of these two groups could not have regenerated in either of these exclusive ways: it must have combined both processes, each with a more restricted scope. The primitive mode of regeneration can therefore have been little more than one of specific tissue-repair, gut repairing gut, and atrium repairing atrium, exactly as in Asymmetron according to Andrews: 'Here, as in the tadpole or salamander, there seems to be a regeneration of each tissue to form new tissue of its own kind' (1893, p. 27. Cf. Franz on Amphioxus, 1925, p. 438).

Let us imagine the primitive Tunicate as a little sac-shaped organism fixed by a short ventral stalk to a creeping stolon, like a creature intermediate between Rhabdopleura and Cephalodiscus, but with a complete gelatinous cuticle and the general organization of a young Ciona. After a period of feeding activity, during which the stalk became filled with reserve food-stuffs, the pharyngeal region died down, leaving only a few patches of undifferentiated material at the base of the former pharynx and atrial cavities. It is well known that these are the sites of active growth in an Ascidian, so that it is precisely here that undifferentiated material is normally concentrated—on either side of the retro-pharyngeal tract in the pharynx, and at the base of each atrium. To meet this periodic phase of atrophy and renewal, short diverticula or pockets from these regions would be a natural provision as in other cases, and would enable the process of regeneration to begin without delay. Judging from Oka's figs. 4 and 6 (1906), it seems probable that in Aphanibranchion (=Diazona?) atrium and pharynx are still regenerated from independent rudiments, although explicit statements on this point are lacking.

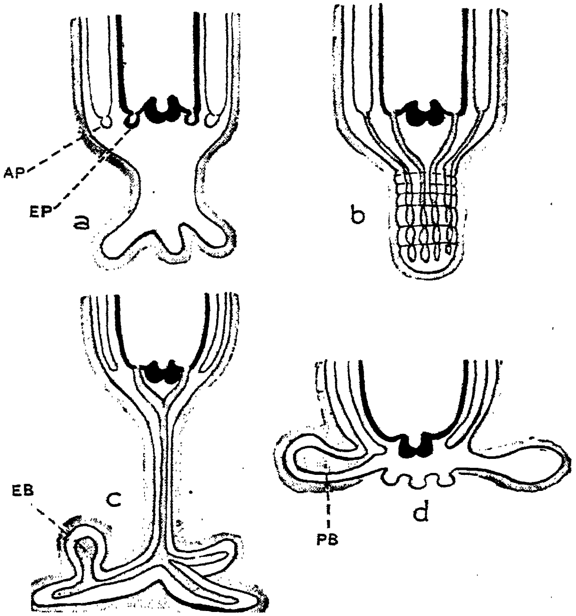
In Clavelina, before the process of degeneration has extended into the abdomen, there is a well-marked stage when the atrium is reduced to a pair of small vesicles alongside a pair of 'epicardial horns' on either side of the degenerating pharynx. If we imagine the stolonial extension of the two epicardia (i.e. the endophragm) to be absent, this condition is strikingly suggestive of the two pairs of regenerative pockets which I have postulated as an early provision for purely thoracic regeneration (see J. S. Huxley, 1926, p. 12, Pl. III, fig. 5 *b*).

From such a stock of fixed, budding, and regenerating Protounicates the Thaliacea would be derivable, possibly through ancestors that, like many Graptolites, were fixed to floating objects, thus facilitating the transition to a free pelagic life. The normal atrophy of the pharynx in old Doliolid nurses and Appendicularians may well be a relic of the periodic degeneration characteristic of their sessile predecessors, the original sequel of rejuvenation having been biologically replaced in Doliolum by an intensification of the budding process, and in Appendicularians by precocity of sexual reproduction.

A provision of regenerative pockets at the junction of thorax and abdomen would yield all the elements required for the complex Thaliacean stolon. The pairs of pharyngeal and peribranchial diverticula, originally developed for use in simple regeneration, could readily extend downwards into the stalk, and thus meet the needs of the stolon, as the ectoderm became too specialized to play its original major part in the budding process.

In support of this suggestion, I need only point to the salient facts in the history of the Thaliacean stolon. In Pyrosoma, which admittedly retains the most primitive condition, the budding process is entirely based on the co-operation of specific organ-regenerators: 'Alle Stolostränge . . . sind abgeschnürte Fortsätze der entsprechenden Organe des Muttertieres . . . um als "Stränge" in den Stolo einzutreten und wieder die entsprechenden Organe der neuen Knospe zu bilden' (Neumann in Bronn, 1913, p. 150). In Salpa, although certainty is out of the question, it is probable that the specificity of some of the stolonial strands has broken down: the endodermal tube is derived from the pharynx and reproduces a pharynx; but, if Korotneff is right, it also proliferates the peribranchial tubes, and has thus enlarged its primitive scope, like the epicardium of Endoblastica (Korschelt and Heider, 1910, p. 780). Finally in Doliolum, if one dare refer to so controversial a type, it would seem that all remnants of original specificity have disappeared: the epicardial tubes, no longer united, give rise apparently to the reproductive organs, while the cloacal tubes, on the other hand, double inwards on themselves to form secondary strands which unite to constitute the pharynx (Neumann; cf. Korschelt and Heider, l. c., p. 809). Thus in Thaliacea the constituents of the stolon may be regarded at the outset as special regenerators of the particular organs from which they arose, but in the course of evolution they have acquired formative powers having little or no relation to their morphological origin. This means that they have become indifferent, or generalized, budding organs, controlled, we know not how, by epigenetic factors. The ultimate result of such a change should be a simplification of machinery by a reduction of elements, but Salpa and Doliolum show no numerical reduction. Nevertheless a qualitative, if not a quantitative, change in this direction is recognizable even in them, since certain of the constituent strands appear to have taken over a preponderating role in the budding process. It can hardly be

TEXT-FIG. 13.



To illustrate the different modes of budding in Tunicata, and their relation to thoracic regeneration. *a*, hypothetical Prototunicate with regenerative discs or pockets at base of pharynx and atrial cavities; *b*, Protothaliacean showing extension of both pairs of pockets into stolon (amphiblastic); *c*, Endoblastic Ascidian, retaining endodermal diverticula alone (epicardia) and erect attitude; *d*, Periblastic Ascidian, retaining peribranchial diverticula alone, with broad base of attachment. On the right a marginal process (ampulla), on the left a bud, in the centre the three primitive adhesive papillae.

AP, atrial or peribranchial pocket; EP, endodermal or pharyngeal pocket (primitive epicardium); EB, epicardial bud; PB, peribranchial bud.

without significance (if the facts have been correctly determined) that in one of these cases the Endoblastic element, and in the other the Periblastic, shows signs of predominance. Apparently pelagic life has not favoured any great development of these tendencies to concentration, for the full expression of which certain conditions of fixation seem to have been required, as will appear later.

From the same Prototunicate stock the Endoblastic Ascidians would be derivable by retention of the median stalk of fixation as the apex of the stolon, and of the pharyngeal diverticula (epicardia) which ran into it, but the lateral or peribranchial diverticula were dispensed with. Originally, as in the larva, the intestinal loop would lie behind the stalk of fixation, and somewhat dorsally, as in Pterobranchia, so that the stalk itself and the pharyngeal diverticula into it may have been quite short. A remnant of this condition persists in such short-bodied forms as Perophora; but in general those Phlebobranchiate Ascidians which have dispensed with budding tend to lie over on their right (Corella) or left side (Ascidia), which gives them a broader area of attachment, and facilitates an extension of the pharynx behind the intestinal loop altogether. On the other hand, the Haplobranchiate Ascidians, which are plainly derivable from a primitive Cionid stock, but have developed, rather than reduced, their early powers of budding, are all vertically elongated, and the intestinal loop has descended into the stalk of fixation in a manner very similar to that exemplified in Phoronis, though less abruptly. This descent of the gut has converted the proximal part of the stolon into a secondary abdominal cavity, which has to be traversed by the epicardia—or the endophragm resulting from their fusion—before they can reach the base of attachment where budding actually proceeds; and the extension of the epicardia has naturally been utilized for the more efficient separation of afferent and efferent blood-channels both in the stolon itself and in the abdominal cavity formed from its proximal portion. Such a use involves no specialization of its tissue.

Thus on the assumption of a primitive relation of stolon to body similar to that in Pterobranchia, every stage in the further evolution of parts characteristic of Endoblastic Tunicates can be satisfactorily accounted for, both morphologically and physiologically, whether budding has been retained as in the

Haplobranchs or discarded in favour of greater individual development as in the Phlebobranchs. In the latter section the characteristic 'test-vessels' of Ciona with their dividing septum represent simply an adaptation to somatic ends of the former stolon with its epicardial axis, while the 'double-vessels' of Phallusia and other simple Ascidians (Seeliger in Bronn, pp. 246, 552, 575), which have a similar origin and distribution, can only consistently be regarded as the same vessels simplified by failure of the epicardium to develop, and readapted for the double circulation by longitudinal constriction of their walls. That the epicardial septum should have replaced such an arrangement is unthinkable.

In contrast to the Endoblastica, the Periblastica, though derivable from the same original stock, have undergone a secondary change in the conditions of fixation which has profoundly affected their processes of growth, budding, and general symmetry of organization. It may be roughly described as a change from the centripetal concentration of organs to their centrifugal dispersal, and is typically associated with a flattening of the body instead of its vertical elongation. The most primitive stock on the whole consists of the Botryllids which still retain the original median group of larval suckers, but these are combined with a peripheral ring of ectodermal papillae, which in the remaining groups tend to replace the original suckers altogether. The result of this arrangement is that on fixation the original median area of attachment, which forms the extremity of the stolon in Endoblastic Ascidians, is enclosed and completely buried within the larger disc of adhesion outlined by the peripheral ring of papillae, so that budding from a median stolon is effectively precluded. (Excellent figures of this condition for Botryllus and Styelopsis in Damas, 1904, Pl. xxi.) It is natural, therefore, that with the loss of the median stolon should go also the loss of the inner or pharyngeal pair of bud-diverticula, while the outer or peribranchial pair are retained in connexion with the lateral or parietal budding that has been substituted for median budding.

It is remarkable that a ring of ectodermal papillae surrounding the primitive stalk of fixation is also found in the larvae of many Didemnids (Lahille, l. c., fig. 64; Oka and Willey, 'Quart. Journ. Micr. Sci.', xxxiii, fig. 7), and doubtless serves the same function in widening the base of fixation. They are not necessarily homologous in the strict sense, because in each case they represent simply a precocious development of ectodermal prolongations characteristic of the adult phase, and mainly subservient to test-production. But it is interesting to note that the absence of a median stolon in Didemnids is explicable as a consequence of the same larval phenomenon as in Periblastica, and may well have been the chief factor in bringing about the remarkable modification of epicardial budding which is a distinguishing feature of this family. If there are any to whom this mixture of budding and regeneration seems to present an incipient stage in the development of budding from regeneration, they should refer to Brien's illuminating study of the budding (abdominal strobilization) of Aplidium (1925), which is shown to form 'le pont réunissant la blastogénèse des Aplidiens à celle des Didemnidae', and carries the story back to the post-abdominal (stolonial) fission of Polyclinidae, in which, as in the stolonial budding of Clavelina, the epicardium bears the entire burden of organ-formation.

The differences between endoblastic and periblastic budding are undoubtedly substantial, and may seem at first sight to imply complete independence of origin, or at least to involve a sharp mutation. In the preceding sketch, though necessarily condensed, I have tried to show that a complete continuity of budding may have been preserved through all the main lines of Tunicate evolution, even from pre-Tunicate ancestors, in spite of great changes in the actual mode of budding. In this connexion we have to remember the extraordinary adaptability of the budding process, as of regeneration itself. It is difficult enough to trace the evolution of a particular organ, and impossible fully to understand it, without considering it in relation with the evolution of the body generally. This difficulty is increased tenfold in the case of the budding process, and renders it essential that it should be considered in connexion with the general phyletic relations of the groups involved. As Caullery has put it: 'Le bourgeon porte au plus haut point l'empreinte d'une épigénèse, c'est-à-dire d'une évolution dépendant avant tout des conditions environnantes' (l. c., p. 137). Hence we find great differences in the mode of budding even in genera so closely related as Clavelina and Distaplia, although in this case there can be no question as to the continuity of budding itself. In Clavelina there is a median stolon traversed by a single epicardial septum, and the buds arise leisurely after fixation as outgrowths from it. In Distaplia there is neither stolon nor septum, but a pair of minute epicardial diverticula, from one of which alone a vesicle is constricted, which clothes itself with ectoderm as it migrates through the parental skin, and divides into fragments, each of which develops into a complete zooid. This is not parietal budding, but it is a step in that direction, for the median stolon has gone, and the source of the bud is distinctly lateral. What are the 'environing conditions' that have provoked this change? Simply the formation of a solid colony, instead of a diffuse one, and the provision of yolky eggs. So we get precocity of bud formation in the larva instead of in the adult; early detachment under cover of the parental test; and finally fragmentation from a single epicardium before the latter has fused with its fellow.

Again, none can question the continuity of budding in the Thaliacea, although there are considerable differences between Pyrosoma, Anchinia, Doliolum, and Salpa in the constitution of their stolon and the developmental processes in the buds.

With these examples before us I submit that the peculiarities of budding in the Periblastica are perfectly consistent with the descent of this group from early Tunicates still provided with a ventral median stolon, the change in their mode of budding being adaptive to recognizable changes in their mode of fixation and orientation. That Molgulids retain modified vestiges of the original paired epicardia has already been mentioned (Damas, 1902).

It results from this study that epicardial and peribranchial outgrowths subservient to budding have probably arisen within the Tunicate group itself and are derivable from corresponding pockets of undifferentiated material which originally served as regenerative centres for the renewal of pharynx and atrium after degeneration. Assuming that the ventral stalk of fixation was already in existence in the ancestors of Tunicates, and was the site of budding processes after fixation, it became transformed into the stolo prolifer of Tunicata by the extension into it of prolongations from the regenerative pockets just mentioned. The earliest type was probably ‘amphiblastic’ with paired diverticula both from pharynx and atrium, a type which has survived only in the pelagic Thaliacea, but with loss of the terminal suckers of fixation. In the Ascidiacea, however, the diverticula were soon reduced to one pair or the other, the Endoblastica retaining the pharyngeal outgrowths (as ‘epicardia’) in conjunction with terminal suckers and the adoption of an upright attitude with extended intestinal loop (secondarily lost in Phlebobranchia which discarded budding altogether), while the Periblastica retained only the peribranchial pockets for lateral budding in adaptation to the lateral extension of a compact body, with widened base of fixation, and more or less horizontal attitude. Budding once established from the posterior peribranchial diverticula, there was of course no obstacle to its extension over the whole parietal wall of the peribranchial cavity, which undergoes no special differentiation.

In many types, both of Endoblastica and Periblastica, budding has disappeared altogether, the loss of budding bringing in its train increase of size, longer duration of life, and greater complexity of pharyngeal structure (Ascidiidae, Molgulidae, Cynthiidae); but the fact that the families of large solitary Ascidians are each related intimately to small, budding forms of more primitive structure, points strongly to the absence of budding in those cases as secondary, e.g. Rhopalaea leading to Ciona, Perophora to Ascidia, Botryllus to the Molgulids, and Polystyelids to Cynthiids. On the current view, according to which the solitary Ascidians are primitive, and budding is regarded as a secondary acquisition, the existence of such structures as the endodermal septum of the test-vessels in Ciona remains completely unintelligible.

In conclusion, it is desirable to see how far this independent study of budding in Tunicates accords with the conclusions already reached with regard to Amphioxus. We have seen that in the character of its U-shaped gill-slits and endostyle, as well as in its protonephridia, it retains more primitive features than any Tunicate, but that in its elongated form, multiplication of gill-slits, peculiar atrium, and lack of fixation and budding, it has deviated from the primitive Protochordate stock, which in these respects resembled the Tunicata. The common ancestor was essentially a small stalked Ascidian with the pharynx of a young, but symmetrical Amphioxus having only two to three pairs of U-shaped gill-slits. To these characters we now add that the stalk of fixation arose ventrally behind the endostyle, and at first contained mesoderm only, as in Pterobranchia. Subsequently, but before the origin of any existing type of Tunicate, there ran into it also two pairs of regenerative diverticula from the pharynx and lateral atria respectively. It is to this stage in the phylogeny that we refer the origin of Amphioxus, of which it may be regarded as the paedomorphic representative. It is therefore quite consistent to find that Amphioxus possesses a vestige of the epicardial diverticula as previously suggested, in its club-shaped gland. What is more remarkable, perhaps, and certainly unexpected, is to find that Lankester's pair of 'atrio-coelomic funnels' arise from the atrium in exactly the spot where the pair of peribranchial diverticula into the stolon ought to arise if they had persisted, viz. alongside the hinder extremity of the pharynx where the 'base' of the lateral atria would be in a primitive sessile Tunicate! This coincidence is all the more striking because in Amphioxus the atrium extends far behind this primitive limit, like the perivisceral extensions in a Botryllus, so that one can hardly avoid the conclusion that the coincidence is significant. Amphioxus in that case possesses homologues of the four principal constituents of the Thaliacean stolon, and incidentally corroborates the theory of the primitive nature of that type of stolonial constitution. The extreme dislocation between the pharyngeal and the peribranchial pair, with the whole length of the pharynx between them, is readily explained on the one hand by the precocious specialization of the pharyngeal pair and its fixation anteriorly in the larva as the club-shaped gland, and on the other by the retarded development of the atrium. There is, of course, nothing but circumstantial evidence to support the homology suggested for the atrial funnels, but, in the absence of any evidence of a function for these structures, it is permissible to regard them provisionally as mere vestiges. It should be added that Lankester's expectation of the existence of a coelomic pore at the apex of the funnels has been definitely negatived by Franz (1925, p. 449) after special investigation.

POSTSCRIPT.

Since this essay was sent to press two recent papers by Van Wijhe (1926 and 1928) have come to my notice which bear closely on some of the problems I have been discussing. Van Wijhe has observed that the larva of Amphioxus, during its asymmetrical growth-period, is capable of temporary fixation, as previously noted by Orton (1914, *b*); but, whereas Orton considered the means of attachment to be the secretion of the club-shaped gland, Van Wijhe traces it to a pair of glandular patches near the anterior ends of the future 'atrial folds', and identifies them, together with the unpaired 'mandibular papilla' in front of the mouth, as homologues of the three adhesive papillae of Ascidian tadpoles. If this comparison can be sustained, it will obviously add support to the views here maintained, since the papillae in any particular Ascidian are all similarly constituted, while in Amphioxus their supposed homologues are distinctly heteromorphic, and the posterior pair become drawn out into the long 'tape-like' stripes of 'glassy' cells which have been so often figured (and variously interpreted) along the edges of the metapleural folds (Lankester and Willey, 1890, p. 466, figs. 1, 2, 7, 13, 14; MacBride, 1900, p. 356, figs. 9, 10; Gibson, 1910, p. 228, figs. 8–12).

Nevertheless, it cannot be assumed without further investigation that the arrangement of these papillae in a triangle, with one in front and two behind, is either typical or primitive in Ascidians. In Botryllus and Corella, apparently also in Distaplia and Clavelina, the condition is just the reverse (viz. a pair of papillae in front and a median papilla behind), and in Amaraecium all three papillae lie consecutively in a median row. The innervation of the papillae in Botryllus rather indicates that, morphologically, one left-sided papilla is balanced by two on the right side—a condition also suggested by Kupffer's figure of the larva of Ciona. As this condition is completely reversed in Amphioxus, it will plainly be no easy matter to establish individual homologies between them.

The relation of the posterior 'glandular' patches to the 'pterygial folds', however, has already led Van Wijhe to assert the complete independence of the atrial cavities of Amphioxus and the Tunicata. A great deal depends therefore on the cogency of Van Wijhe's evidence, of which a fully illustrated account is promised.

The further discovery is announced by Van Wijhe (1926) of a transitory funnel-like communication between the pre-oral (buccal) cavity and the anterior extremity of the pharynx, and this duct is now substituted for Hatschek's pit as the 'autostome' or primitive mouth. From the brief details given in the preliminary note, it is impossible to assess the significance of this new aperture. From the fact that it arises late and is said to be 'filled with a slimy mass . . . most likely derived from the pre-oral organ', the duct may be merely the result of a closure of the 'mandibular groove' between Hatschek's pit and the mouth—in which case it would be a secondary passage, comparable to a nasal duct, rather than the relic of a 'primitive mouth'. But it may be recalled that Goodrich (1909, p. 197) has already reported the existence of a communication in one of his larvae between the buccal cavity and the duct of Hatschek's nephridium (indirectly therefore with the pharynx), and it will be of great interest to learn whether this new duct has any relation to that isolated organ.

In his latest paper (1928, p. 997) Van Wijhe has anticipated me in drawing attention to the contrast between Appendicularians and other Tunicates in regard to the twist of the intestinal loop, but by overlooking the exceptional cases of Doliolum and Anchinia he has been unfortunately led into an untenable hypothesis. Regarding the mouth and club-shaped gland as the first pair of gill-slits in Amphioxus, and homologous with the pair of spiracles in Appendicularians, he now suggests that the right intestine of Appendicularians and the left intestine of other Tunicates together represent the second pair of gill-slits in Amphioxus, of which the left can be regarded as more potent than the right! The existence of Doliolum, however, with its median gut and anus, destroys the basis of this curious proposition, since it prohibits the 'sharp division' of Tunicates into 'Dextricolica' and 'Laevicolica' on which Van Wijhe was relying. I may add that, whatever may be thought of the eccentric mouth of the larval Amphioxus, there is really no adequate reason for disputing the homology of the Tunicate intestine with that of Amphioxus. The blastopore is closed over alike in the embryos of both types, and there is a secondary anal outgrowth in Amphioxus as in Ascidians, so that the difference between them in this respect is only one of degree. If a gill-slit were needed to make an outlet for one, it would be just as necessary for the other. The presence of 'subchordal' cells in the Ascidian tail does not affect this question if I am right in my conclusion that this region is represented in Amphioxus only by the greatly reduced larval fin (p. 155).

BIBLIOGRAPHY.

Agassiz, A. (1873).—" The History of Balanoglossus and Tornaria ", 'Mem. Amer. Acad. Arts and Sci.', ix.

Andrews, E. A. (1893).—" On Asymmetron lucayanum ", 'Stud. Biol. Lab. Johns Hopkins Univ.', v.

Assheton, R. (1895).—" Notes on the Ciliation of the Ectoderm of the Amphibian Embryo ", ' Quart. Journ. Micr. Sci.', xxxviii.

Balfour, F. M. (1895).—' Treatise on Comparative Embryology ' (2nd ed.).

Barrois, J. (1885).—" Rech. sur . . . l'Anchinie ", ' Journ. Anat. Physiol. Paris ', xxi.

Bateson, W. (1885).—" The Later Stages in the Development of Balanoglossus Kowalevskii ", ' Quart. Journ. Micr. Sci.', xxv, Suppl.

—— (1886).—' The Ancestry of the Chordata,' ibid., xxvi.

Brachet, A. (1921).—' Traité d'Embryologie des Vertébrés.'

Brien, P. (1925).—' Contribution à la blastogénèse des Tuniciers (Aplidium zostericola) ", ' Arch. de Biol.', xxxv, and 1927, xxxvii.

Brooks, W. K., and Cowles, R. P. (1905).—" On Phoronis architecta ", Mem. Nat. Acad. Sci. Philadelphia ', x.

Bury, H. (1889).—"Studies in the Embryology of the Echinoderms", ' Quart. Journ. Micr. Sci.', xxix.

—— (1895).—" The Metamorphosis of Echinoderms ", ibid., xxxviii.

Caullery, M. (1895).—" Contribution à l'étude des Ascidies composées ", ' Bull. Sci. France et Belg.', xxvii.

Damas, D. (1899).—" Les Formations épicardiques chez Ciona intestinalis ", ' Arch. de Biol.', xvi.

—— (1902).—" Rech. sur le développement des Molgules ", ibid., xviii.

—— (1904).—" Contr. à l'étude des Tuniciers (1. Segmentation de la queue des Appendiculaires ; 2. Branchie des Tuniciers ; 3. Larve de Distaplia) ", ' Arch. de Biol.', xx.

De Beer, G. R. (1926).—' The Comparative Anatomy, Histology, and Development of the Pituitary Body.'

Delsman, H. C. (1910).—" Beitr. z. Entwicklungsgeschichte v. Oikopleura ", ' Verh. Rijksinstituut Onderz. Zee ', iii.

—— (1912).—" Weitere Beob. üb. die Entwicklung v. Oikopleura ", ibid., xii.

—— (1921).—" The Ancestry of Vertebrates ", ' Natuurkundig Tijdschrift voor Nederlandsch-Indië ', lxxxi.

Eastman, C. R. (1913).—' Zittel's Text-book of Palaeontology,' i.

Fol, H. (1872).—" Études sur les Appendiculaires ", ' Mém. Soc. de Phys. et d'Hist. Nat. de Genève ', xxi.

—— (1876).—" Ueber die Schleimdrüse oder den Endostyl der Tunicaten ", ' Morph. Jahrb.', i.

Fowler, G. H. (1905).—" Notes on Rhabdopleura normani ", ' Quart. Journ. Micr. Sci.', xlviii.

Franz, V. (1922).—" Kurzer Bericht üb. systematische Acranierstudien ", ' Zool. Anz.', liv.

—— (1922).—" System. Revision d. Akranier ", ' Jen. Zeit.', lviii.

—— (1924).—" Lichtversuche am Lanzettfisch . . .", ' Wiss. Meeresuntera. (N.F.) Helgoland ', xv.

Franz, V. (1925).—" Morph. u. Ont. Akranierstudien ", ' Jen. Zeit.', lxi.

Fritsche, E. (1910).—"Die Entwicklung d. Thymus bei Spinax niger ", 'Jen. Zeitschr. Wiss.', xxxv.

Garstang, Sylvia and W. (1926).—" On the Development of Botrylloides and the Ancestry of Vertebrates (Preliminary Note) ", ' Proc. Leeds Phil. Soc.', i. Also (1928) ' Quart. Jour. Micr. Sci.', lxxii.

Garstang, W. (1892).—" On the Development of the Stigmata in Ascidians ", ' Proc. Roy. Soc.', li.

—— (1893).—" On some Bipinnariae from the English Channel ", ' Quart. Journ. Micr. Sci.', xxxv.

—— (1893 *b*).—" Note on Salensky's Account of the Development of the Stigmata in Pyrosoma ", ' Trans. Biol. Soc. Liverpool '.

—— (1894).—" Preliminary Note on a new Theory of the Phylogeny of the Chordata ", ' Zool. Anzeiger ', xvii.

—— (1895).—" Outlines of a new Classification of the Tunicata ", ' Rep. Brit. Ass. Ipswich '.

—— (1921).—" The Theory of Recapitulation—a critical restatement of the Biogenetic Law ", ' Linn. Soc. Journ. (Zool.) ', xxxv.

Gibson, H. O. S. (1910).—" The Cephalochorda : Amphioxides ", ' Trans. Linn. Soc.' (2), xiii.

Gilchrist, J. D. F. (1915).—" Observations on the Cape Cephalodiscus and some of its early stages ", ' Ann. Mag. Nat. Hist.', (8) xvi.

—— (1917).—" On the Development of the Cape Cephalodiscus ", ' Quart. Journ. Micr. Sci.', lxii.

—— (1919).—" Reproduction by Transverse Fission in Phoronopsis ", ibid., lxiii.

Goldschmidt (1905).—" Amphioxides ", ' Wiss. Ergebn. Deutschen Tiefsee-Expedition ', xii.

—— (1906).—" Amphioxides und Amphioxus ", ' Zool. Anz.', xxx.

Goodrich, E. S. (1909).—" On the structure of the Excretory Organs of Amphioxus ", ' Quart. Journ. Micr. Sci.', liv.

—— (1917).—" ' Proboscis Pores ' in Craniate Vertebrates, a suggestion concerning the Premandibular Somites and Hypophysis ", ibid., lxii.

Grave, C. (1921).—" Amaroucium constellatum. II. Structure of the Tadpole Larva ", ' Journ. Morph.', xxxvi.

—— (1926).—" Molgula citrina. Activities and Structure of the Free-swimming Larva ", ibid., xlii.

Grave, C., and Woodbridge, H. (1924).—" Botryllus schlosseri : the Behaviour and Morphology of the Free-swimming Larva ", ibid., xxxix.

Harmer, S. F. (1887).—" Cephalodiscus ", ' " Challenger " Reports ', xx.

—— (1905).—' The Pterobranchia of the Siboga Expedition,' xxvi bis.

—— (1917).—" On Phoronis ovalis ", ' Quart. Journ. Micr. Sci.', lxii.

Hecht, S. (1918).—" The Physiology of Ascidia atra ", ' Journ. Exp. Zool.', xxv.

Herdman, W. A. (1883).—" On the Homology of the Neural Gland in the Tunicata with the Hypophysis Cerebri ", ' Proc. Roy. Soc. Edin.', xii.

—— (1904).—" Tunicata ", ' Cambridge Natural History '.

Hjort, J. (1896).—" Germ-layer Studies based upon the Development of Ascidians ", ' Norweg. North Atlantic Exped.', 1876–8.

Huntsman, A. G. (1913 *a*).—" Protostigmata in Ascidians ", ' Proc. Roy. Soc.', lxxxvi.

—— (1913 *b*).—" On the Origin of the Ascidian Mouth ", ibid.

Huxley, J. S. (1923).—" Dedifferentiation in Clavelina ", ' Quart. Journ. Micr. Sci.' lxvi.

Ihle, J. E. W. (1913).—" Die Appendicularien ", Spengel's ' Ergebnisse u. Fortschritte der Zoologie ', iii (Kritik).

—— " Ueber den Endostyl . . . der Appendicularien ", ' Zool. Anz.', xxxi.

Julin, Ch. (1904 *a*).—" Rech. sur la phylogenèse des Tuniciers. Développement de l'appareil branchial ", ' Zeit. f. wiss. Zool.', lxxvi.

—— (1904 *b*).—" Rech. etc. Archiascidia neapolitana, nov. gen. nov. sp.", ' Mitth. Zool. Stat. Neapel ', xvi.

Kemna, Ad. (1905).—" L'Origine de la corde dorsale ", ' Ann. Soc. Roy. Zool. et Malacol. de Belgique ', xxxix.

Kerb, H. (1908).—" Zur Frage der Ueberwinterung der Ascidien ", ' Arch. mikr. Anat.', lxxii.

Kerr, J. Graham (1919).—' Text-book of Embryology ', II. Vertebrata.

Korotneff, A. (1884).—" Die Knospung der Anchinia ", ' Zeit. f. wiss. Zool.', xl.

—— (1891).—" La Dolchinia mirabilis ", ' Mitth. Zool. Stat. Neapel ', x.

—— (1904).—" Über d. Polymorphismus v. Dolchinia ", ' Biol. Centr.', xxiv.

Korschelt, E., and Heider, K. (1893).—' Lehrb. d. vergl. Entwick. d. wirbellosen Thiere ', iii.

—— (1910).—Ditto, iv, " II. Ungeschlechtliche Fortpflanzung ".

Kowalevsky, A., et Barrois, J. (1883).—" Matériaux pour servir à l'hist. de l'Anchinie ", ' Journ. Anat. Physiol. Paris ', xix.

Lahille, F. (1890).—' Recherches sur les Tuniciers.'

Lankester, E. R. (1884).—" Rhabdopleura ", ' Quart. Journ. Micr. Sci.', xxiv.

—— (1889).—" Amphioxus ", ibid., xxix.

—— (1898).—" Note on the Development of the Atrial Chamber in Amphioxus ", ibid., xl.

Lankester, E. R., and Willey, A. (1890).—" The Development of the Atrial Chamber of Amphioxus ", ibid., xxxi.

Lefevre, G. (1898).—" Budding in Perophora ", ' Journ. Morph.', xiv.

—— (1897).—" Budding in Ecteinascidia ", ' Anat. Anz.', xiii.

Lohmann, H. (1896).—" Die Appendicularien der Plankton-Expedition ", ' Ergebnisse ', vol. 2, E, c.

—— (1899).—" Das Gehäuse d. Appendicularien ", ' Schr. Naturw. Ver. Schleswig-Holstein ', xi (abstract in ' Zool. Anz.', xxii).

Lohmann, H. (1902).—" Neue Unters. üb. den Reichthum des Meeres an Plankton ", ' Wiss. Meeresuntersuch. Kiel ' (N.F.), vii.

—— (1905).—" Die Appendicularien des arkt. u. antarkt. Gebiets, &c.", ' Zool. Jahrb.', Suppl., viii.

—— (1909).—" Die Gehäuse u. Gallertblasen der Appendicularien, &c.", ' Verh. Deutsch. Zool. Ges.'

Lohmann und Bückmann (1926).—' Die Appendicularien der Deutschen Südpolar-Expedition, 1901–3,' xviii, pp. 63–231.

Longchamps, M. de Selys (1901).—" Développement du cœur, du péricarde et des épicardes chez Ciona intestinalis ", ' Arch. de Biol.', xvii.

—— (1900).—" Développement de la branchie chez Corella ", ' Arch. de Biol.', xvii.

—— (1902).—" Rech. sur le développement des Phoronis ", ' Arch. de Biol.', xviii.

—— (1903).—" Ueber Phoronis u. Actinotrocha ", ' Beitr. z. Meeresfauna v. Helgoland ', xiv.

—— (1915).—" Autotomie et régénération des viscères chez Polycarpa tenera ", ' Comptes Rendus ', t. 160.

—— (1916).—" Les Statoblastes de Stolonica socialis ", ' Bull. Soc. Zool. France ', xli.

—— (1917).—" Sur le bourgeonnement des Polystyélinés ", ' Bull. Sci. France et Belgique ', l.

MacBride, E. W. (1894).—" Review of Spengel's Monograph of Balanoglossus ", ' Quart. Journ. Micr. Sci.', xxxvi.

—— (1898).—" The Early Development of Amphioxus ", ibid., xl.

—— (1900).—" Further remarks on the Development of Amphioxus ", ibid., xliii.

—— (1909).—"The Formation of the Layers in Amphioxus, &c.", ibid., liv.

—— (1914).—' Text-book of Embryology,' I. Invertebrata.

—— (1926).—" The Recapitulation Theory ", ' Science Progress '.

Martini, E. (1909).—" Stud. über die Konstanz histologischer Elemente. II. Fritillaria pellucida ", ' Zeit. f. wiss. Zool.', xciv.

—— (1909 *b*).—" Ueber Eutelie u. Neotenie ", ' Verh. d. Deutsch. Zool. Gesellschaft ', xix, pp. 292–9.

—— (1909 *c*).—"Ueber die Segmentierung des Appendicularienschwanzes", ibid., pp. 300–7.

—— (1910).—" Weit. Bemerkungen üb. die sogenannt. metamere Segmentierung d. Appendicularienschwanzes ", ' Zool. Anz.', xxxv.

Masterman, A. T. (1897).—" On the Diplochorda. I. Actinotrocha ; II. Cephalodiscus ", ' Quart. Journ. Micr. Sci.', xl.

—— (1898).—" On the further Anatomy and Budding Processes of Cephalodiscus ", ' Trans. Roy. Soc. Edin.', xxxix.

—— (1900).—" On the Diplochorda. III. Development and Anatomy of Phoronis ", ' Quart. Journ. Micr. Sci.', xliii.

Masterman, A. T. (1903).—" Ditto. IV. Central Complex of Cephalodiscus", ibid., xlvi.

—— (1908).—" Ditto. V. Tornaria ", ibid., lii.

Metcalf, M. M. (1900).—" Notes on the Morphology of the Tunicata ", Spengel's ' Zool. Jahrb., Abth. f. Anat. u. Ont.', xiii.

Morgan, T. H. (1891).—" The Growth and Metamorphosis of Tornaria ", ' Journ. Morph.', v.

—— (1894).—" The Development of Balanoglossus ", ibid., ix.

Neumann, G. (1913).—" Doliolum ", ' Wiss. Ergebn. d. Deutschen Tiefsee-Expedition ', xii.

Oka, A. (1906).—" Aphanibranchion, eine neue Synascidiengattung aus Japan ", ' Annotat. Zool. Japan ', v, no. 5.

—— (1913).—" On Cyathocormus mirabilis ", ' Journ. Coll. Sci. Tokyo ', xxxii.

—— (1926).—" On the mode of Gemmation in Dictyostyela depressa, n. gen. et sp.", ' Proc. Imp. Acad.', ii.

Orton, J. H. (1913).—" The Ciliary Mechanisms on the Gill and the Mode of Feeding in Amphioxus, Ascidians, and Solenomya ", ' Journ. Mar. Biol. Ass.', x. 1.

—— (1914).—" On Ciliary Mechanisms in Brachiopods, &c., and an account of the Endostyle of Crepidula ", ibid., x. 2.

—— (1914 *b*).—" On a Hermaphrodite specimen of Amphioxus, with Notes on Rearing ", ibid., x. 3.

Perrier, E. (1897).—' Traité de Zoologie.' Also (1898) ' C. R. Acad. Sci.', cxxvi.

Ritter, W. E. (1896).—" Budding in Comp. Ascidians based on studies on Goodsiria and Perophora ", ' Journ. Morph.', xii.

—— (1903).—' The Structure and Affinities of Euherdmania claviformis, &c.', Mark Anniversary Volume.

Ritter, W. E., and Davis, B. M. (1904).—" Studies on the Ecology, Morphology, and Speciology of the Young of some Enteropneusta of Western North America ", Univ. of California Publications, ' Zool.', i, no. 5.

Salensky, W. (1893).—" Ueber das Nervensystem d. Larven und Embryonen von Distaplia magnilarva ", ' Morph. Jahrb.', xx.

—— (1903).—" Études anatomiques sur les Appendiculaires ", ' Mém. Acad. Imp. Sci. St. Pétersbourg ', (8) xiii.

Schepotieff, A. (1904).—" Zur Organisation von Rhabdopleura ", ' Bergens Museum Aarbog '.

—— (1907).—" Knospungsprozess u. Gehäuse v. Rhabdopleura ", ' Zool. Jahrb., Anat.', xxiv.

—— (1909).—" Die Pterobranchier des Indischen Ozeans ", ' Zool. Jahrb., Abth. Syst.', xxviii.

Sedgwick, A. (1909).—' Students' Text-book of Zoology,' iii.

Seeliger, O. (1895).—" Die Pyrosomen ", ' Ergebn. d. Plankton-Exped.', ii, E.

Seeliger, O. (1900).—" Einige Bemerkungen über den Bau des Ruderschwanzes der Appendicularien ", ' Zeit. wiss. Zool.', lxvii.

—— (1893–1907).—" Tunicata ", ' Bronn's Klassen u. Ordnungen des Tier-Reichs ', iii, Suppl.

Semon, R. (1888).—" Die Entwickelung der Synapta digitata ", ' Jen. Zeit. f. Naturwiss.', xxii.

—— (1891).—" Zur Morphologie der bilateralen Wimperschnüre der Echinodermenlarven ", ' Jen. Zeit.', xxv.

Smith, K. M., and Newth, H. G. (1917).—" Note concerning the Collar Cavities of the Larval Amphioxus ", ' Quart. Journ. Micr. Sci.', lxii.

Spengel, J. W. (1893).—" Die Enteropneusten ", ' Fauna u. Flora des Golfes von Neapel ', no. 18.

Stendell (1914).—" Betracht. üb. die Phylogenesis der Hypophysis cerebri nebst Bemerkungen üb. den Neuroporus der Chordonier ", ' Anat. Anz.', xlv.

Stiasny, G. (1914).—" Studien üb. die Entw. d. Balanoglossus clavigerus. I. Die Entwicklung der Tornaria ", ' Zeit. f. wiss. Zool.', cx.

—— (1914).—" II. Weitere Entw. bis zur Metamorphose ", ' Mitt. aus der Zool. Stat. Neapel ', xxii.

Stiasny-Wÿnhoff, G., and Stiasny, G. (1927).—" Die Tornarien (Kritik) ", Spengel's ' Ergebnisse u. Fortschritte d. Zool.', vii.

Uebel, E. (1913).—" Oikopleura najadis, n.sp.", ' Zool. Anz.', xli.

Uljanin, B. (1884).—" Doliolum ", ' Fauna u. Flora, Neapel.'

Van Beneden, E., and Julin, Ch. (1887).—" Recherches sur la morphologie des Tuniciers ", ' Arch. de Biol.', vi.

Wijhe, J. W. van (1907).—" Die Homologisirung des Mundes des Amphioxus u. die prim. Leibesgliederung der Wirbelthiere ", ' Petrus Camper ', iv.

—— (1914).—" Stud. ü. Amphioxus. I. Mund u. Darmkanal während der Metamorphose ", ' Verh. Kon. Akad. Wetensch. Amsterdam ', xviii.

—— (1926)—" On the temp. Presence of the prim. Mouth-opening in the Larva of Amphioxus, and the occurrence of three post-oral Papillae, probably homologous with those of the Larva of Ascidians ", ibid., xxix.

—— (1928).—" Obs. on the Adhesive Apparatus . . . in the living Larva of Amphioxus in the growth-period ", ibid., xxx.

Willey, A. (1891).—" The Later Larval Development of Amphioxus ", ' Quart. Journ. Micr. Sci.', xxxii.

—— (1892).—" The Post-embryonic Development of Ciona and Clavelina ", ' Proc. Roy. Soc.', li.

—— (1893).—" Studies on the Protochordata. I. On the Origin of the Branchial Stigmata, &c., in Ciona and Clavelina ", ' Quart. Journ. Micr. Sci.', xxxiv.

—— (1893).—" II. The Development of the Neuro-hypophysial system in Ciona and Clavelina, with an account of the origin of the Sense-organs in Ascidia mentula." "III. On the Position of the Mouth in the Larvae of Ascidians and Amphioxus, and its relations to the Neuroporus", ibid., xxxv.

Willey, A. (1894).—'Amphioxus and the Ancestry of the Vertebrates,' Columbia Univ. Biol. Series.

—— (1897).—" On Ptychodera flava ", 'Quart. Journ. Micr. Sci.', xl.

—— (1898).—" Spengelia, a new Genus of Enteropneusta ", ibid., xl.

—— (1899).—" Remarks on some recent work on the Protochordata, &c.", ibid., xlii.

Reconstruction of Daughter Nuclei and the individuality of Chromosomal Vesicles during Interkinesis.

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With Plates 4 and 5.

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INTRODUCTION.

THE problem of reformation of daughter nuclei has proved to be a very elusive one. Even the more superficial aspects of the telophasic processes are, to some extent, matters of doubt and disagreement. It seems likely that elucidation of the reconstitutional changes might be achieved by studying them without the presupposition that the components of the metaphase chromosome are equally basophilic. As the mass of daughter chromosomes becomes transformed into an interkinetic nucleus one notes the appearance of strands or sheets of achromatic material. Many accounts have been given of this period which strongly indicate that such achromatic sheets are derived from the surfaces of the formed chromosomes. Further, that they serve as bags in which the chromatin of the metaphase chromosome is enclosed.

A brief consideration of the facts which lend support to such a view seems well worth while. In the early blastomeres of animal eggs the anaphase or telophase chromosomes ordinarily increase greatly in size and become much less chromatic than the metaphase chromosome. The imbibition of achromatic material from the cytoplasm is apparently responsible for this. The achromatin first appears as small, scattered globules which increase in number until the interior of the chromosome, now a chromosomal vesicle, becomes a veritable network of chromatin enmeshing spherules of achromatin. This achromatization or alveolization brings into evidence a thin sheath of material bounding the vesicle. The sheaths are, in most cases, no longer demonstrable after the vesicles become contiguous, except in so far as they give origin to the nuclear membrane when in contact with cytoplasm. In one case they have been followed through the resting nucleus (Richards, 1917). Chromosomal vesicles have been noted in animal eggs by a number of investigators, but their history has probably been followed more closely by Richards (1917) than any other. For this reason we will give some of his interpretations. He considers that the metaphase chromosome consists of an achromatic sheath (linin) or sac filled with chromatin, the alveolization of which gives rise to a vesicle whose border is merely the distended sheath of the earlier chromosome. In addition, that the linin of the resting nucleus as well as the nuclear membrane is traceable to the chromosomal sheaths. The resting nucleus, accordingly, would consist of contiguous vesicles, and there would be no interchromosomal channels present.

The unusual size of the vesicles and the resting nucleus in early blastomeres is probably responsible for the fact that reconstruction of nuclei by chromosome vesiculation was first discovered here. Indeed, it was thought for some time that such a method did not exist elsewhere. However, Sutton (1900) found identically the same process in Orthopteran spermatogenesis. His work has since been extended to many grasshoppers by Wenrich (1916) and others. Wenrich also noted the formation of vesicles in somatic mitosis. The vesiculation of telophase chromosomes in somatic mitosis in animals has been observed in Rana pipiens and Mus norvegicus albinus by myself (Kater, 1927 *a*, 1928). It is to be remembered that the above view does not preclude the possibility of amoeboid processes being thrown out by the chromosomes during the period of reconstruction, but it does exclude the possibility of vesicles in the interkinetic nucleus being held apart, as it were, by such pseudopodium-like projections, so that achromatic channels would intervene.

Animal cytologists, for the most part, have come to regard the alveolization of chromosomes as a method of nuclear reconstitution of very frequent occurrence, to say the least; also its corollary that the resting nucleus is composed of contiguous vesicles. On the other hand many plant cytologists, probably the majority, look upon the metaphase chromosome as a more or less homogeneous body, at least not as a mass of chromatin enclosed in a sheath of different constitution. They hold that the reconstruction of daughter nuclei is a process involving not only alveolization of the chromosomes, but also branching and anastomosing of these units. According to their conception the chromosomes first draw together into a compact mass and later loosen up so that cytoplasmic channels intervene between the chromosomes. These channels are bridged by branching and anastomosing of the chromatin. This view has been especially championed by the Gregoirian school of cytologists (Gregoire, 1906; Gregoire and Wygaerts, 1904; Martins-Mano, 1905; Sharp, 1913, 1920, 1926; Robyns, 1924).

Although chromosomal vesicles are not commonly thought to occur in the somatic cells of plants they have been distinctly figured. Wager (1904) in his illustrations of nuclear division in the root-tip of Phaseolus shows vesicles as distinctly as they have ever been seen in early blastomeres of animal eggs (see fig. 29 in his account). The present writer found his illustrations to be wholly justified. And further, traced the linin sheath of the vesicles through interkinesis in this same plant (Kater, 1926). Although Wager was not interested in chromosomal individuality and in his interpretations overlooked it, he merits credit for so accurately figuring his observations that really have a direct bearing on this important subject. The condition in Phaseolus is especially interesting when one recalls that its resting nucleus has been previously described as possessing interchromosomal channels which arose in the telophase (Martins-Mano, 1905).

It seems desirable to the author to investigate the reconstruction of daughter nuclei in some of the plants that have been used by the Gregoirian school with the object of determining whether or not linin can be differentiated in the reconstruction period, and to what extent the condition in certain other plants is comparable to Phaseolus and many animals, to express it conservatively. This will also afford an opportunity to evaluate the theories of telophasic duality.

The very great importance of this problem can readily be recognized. Firstly, its direct bearing on the subject of chromosomal individuality makes it of paramount interest, owing to the fact that our modern theories of genetics rest entirely upon the assumption of chromosomal individuality. In addition, any theories which we might have concerning cell physiology must be based upon a correct idea of the structures involved, if they are to have any semblance of verity.

The investigation described in these pages was begun at St. Louis University and completed in this department. I wish to thank Professor R. B. Wylie for the many courtesies that have been extended me here.

MATERIAL AND METHODS.

Somatic tissue from three plants has been used in this study. They are Solanum tuberosum, Lycopersicon esculentum, and Allium cepa. Attention has been confined to the root-tips as they probably afford a greater variety of nuclear phases for the bulk of tissue used than any other part of the plant. These three plants were selected because of the gradation of chromosome size which they exhibit: Solanum having very small chromosomes, Lycopersicon having medium-sized ones, and Allium having very large ones. With these varieties of chromosomes it seemed that it would be possible to determine to what extent homologies can be drawn between these types and also to what degree the conditions in such representative plants are comparable to that of Phaseolus and most animals.

The root-tips were fixed either in Bouin's picro-formol-acetic or Flemming's strong mixture. Impregnation was done with low melting-point paraffin and embedding in hard paraffin. Sections were cut at 4 micra to 6 micra. The stains employed for most of the sections was iron-alum haemotoxylin counter-stained with light green. Other dyes were found to be of less value, though in toto staining with saturated eosin in 95 per cent. alcohol followed by haematoxylin after sectioning produced very nice results.

SOLANUM TUBEROSUM.

Reconstruction of Daughter Nuclei.—The chromosomes of Solanum are extremely small, as compared to those of most higher plants. According to Lutman (1925) the diploid number is thirty-six. No careful attempt has been made to determine their exact number, but tentative counts would indicate that Lutman's determination is, to say the least, close to correct. In the early anaphase these numerous small chromosomes are rather irregularly arranged in a band as they move towards the poles of the spindle. It is to be recalled that Phaseolus also possesses quite small chromosomes, though not nearly as small as those of Solanum, and in their migration to the poles of the spindle a very orderly arrangement is maintained, their long axes being consistently parallel. The same relationship is still found after they become contiguous.

This is mentioned because it may help to explain the apparent differences in the process of reconstitution in the two plants. Since they do not form a perfectly even band in the anaphase nor are their long axes parallel in Solanum (fig. 1, Pl. 4), they become enmassed at the poles (' tassement polaire ') in a very unorderly fashion. Some of them even seem not to be in contact with the cytoplasm. Rarely the chromosomes form a regular band at the poles (fig. 2, Pl. 4). After arriving at the poles the chromosomes draw together into such a compact mass that individual ones cannot be identified (fig. 3, Pl. 4). The same type of ' tassement polaire ' is formed in Phaseolus, but the chromosomes can be traced by the indentations on the surface of the basophilic mass (Kater, 1926). This is not true for Solanum. It is true that there are numerous indentations on the surface of the chromosomal mass, but the chromosomes are so small and their number so great that any such conclusion would be purely on the basis of homology with the bean.

This fact makes it evident at the outset that it will be impossible to follow the alveolization of individual chromosomes in this plant. However, that does not preclude the possibility of its study throwing much light on the subject of nuclear structure. It would seem that such a ' tassement polaire ' should afford excellent material for determining whether chromosomes draw apart to permit the formation of achromatic channels between them. This is undoubtedly true. In fig. 4, Pl. 4, one notes that the chromosomal masses exhibit a very even outline, and the proximal side is beginning to give evidence of alveolization. Spherical areas are becoming less basophilic, apparently the result of the appearance of many small achromatic spherules throughout their extent. The distal portion of the mass shows no evidence whatever that the chromosomes are becoming ' less closely packed ' (Sharp, 1926), as it is still evenly and deeply basophilic. In addition, the achromatic areas in the proximal sides are not of such a shape that they could well be considered to be slits between adjacent chromosomes.

In some cases the outline of the ' tassement polaire ' is not so even. Projections are occasionally present which appear very much as if they were derived from a part of a chromosome. Such a projection towards the equatorial side is illustrated in one of the daughter nuclei in fig. 5, Pl. 4. This whole body is becoming achromatized, as well as many other areas in both nuclei. The protuberance exhibits about the same nature and degree of chromaticity as the other alveolized areas, and it seems logical to assume that the cause of decreased basophilia is the same in both cases.

It is appropriate to inquire at this time what cause underlies the processes of reconstruction as far as they have been outlined above. There are two features of this process to be explained, namely, the increase of the size of the reconstituting nuclei and the decrease of basophilia in certain areas. Such an increase in size must be at the expense of the cytoplasm, that is, material must be absorbed from the latter. This assumption would explain, at the same time, the decrease of basophilia. The very small spherules that can be seen suspended within the chromatin are entirely achromatic. This would fit in with the above assumption. It seems probable that temporary permeability of the limiting portion of the nucleus to some cytoplasmic constituents is responsible for this absorption.

The last sentence introduces the topic of the limiting boundary of the nucleus. We might emphasize at this point the importance of studying the nuclear changes at the earliest point possible. It is probable that many of the erroneous conceptions of nuclear structure take origin in investigations which disregarded the very early steps in the transformation of chromosomes into an interkinetic nucleus. Such has been shown to be the case with regard to the origin of the nuclear membrane in the previous work on reconstruction in the bean, frog, and rat (Kater, 1926, 1927 *a*, 1928). It was distinctly shown that the nuclear membrane arises from the sheath or limiting membrane of the chromosomes. Let us return to fig. 5. The membrane which is visible over the achromatized areas and especially the projection from one of the daughter nuclei distinctly appears to be continuous with the surface layer of the non-alveolized portions. This is very indicative of a chromosomal-sheath origin for the nuclear membrane, but is not nearly so clear as in the bean, frog, or rat. The evident condition in Solanum, however, is sufficient to entirely justify homology with Phaseolus with the consequent conclusion that the nuclear membrane in the former also arises from that part of the sheath of the chromosomes that happens to be in contact with the cytoplasm.

As the cell progresses farther in division, as evidenced by the formation of the cell plate, the above-mentioned processes continue. The alveolization of some areas is so great that entirely achromatic spots appear. As a consequence of this dechromatization inward extensions from the nuclear membrane become visible (fig. 6, Pl. 4). The illustration just referred to is very similar to fig. 4 of my paper on Phaseolus (1926) except that the outline of the nucleus is perfectly even and the direct indication that such strands are the borders of chromosomal vesicles is not quite so strong. However, the similarity is so striking that we will assume, for the present, that these rather achromatic strands are the vertical walls of two adjacent chromosomal vesicles. The nuclei at this stage show a decided tendency to have the non-alveolized portions of chromatin collected into one or a few clumps.

Further changes resulting in a return to the resting condition consist in increasing the extent and degree of alveolization and a rearrangement of the densely chromatic particles to form the nucleolus. In fig. 7, Pl. 4, the daughter nuclei are divided into a number of compartments by the sheets of linin and the larger non-alveolized masses of chromatin show a tendency to come together. Fig. 8, Pl. 4, shows the larger masses of chromatin lining up in the nuclei, preparatory to joining. Shortly after this stage the cell plate is completed and the remains of the spindle disappear (fig. 9, Pl. 4). At this time the nucleolus is formed, arising by the juncture of a number of non-alveolized chromatin masses, and the vesicles have become so rearranged that their sheaths radiate about the nucleolus. The vesicles contain numerous small granules of chromatin.

Before leaving the topic of reconstitution of daughter nuclei it is well to summarize the steps in the transformation of a chromosome into a chromosomal vesicle. As mentioned above, when the chromosome first begins to take up achromatic material the chromatin appears to be the continuous phase while the achromatin is suspended in the form of minute spherules. The somewhat dechromatized areas in figs. 4 and 5 are of this nature. The continuation of alveolization reverses this condition. For example, in figs. 7, 8, and 9 the achromatin is continuous and the chromatin takes the form of granules suspended in the former. Whether or not this is a true reversal of phases cannot be stated. It seems possible that the preparation of tissue for study involving fixation, &c., might break delicate sheets of chromatin connecting the larger granules. Such an assumption would relegate this apparent reversal of phases to the realm of artifact. On the other hand, we know that a true reversal of phases is possible in some emulsions. Such may be the case here, but, at the present time, we must leave this feature unsolved.

Even as early as the stage represented in fig. 9 the nucleolus is slightly less chromatic than the metaphase chromosome. The nucleolus here is comparable to the alveolized areas in fig. 4. That this body is typically alveolized in the same manner as the reticulate portion of the nucleus, though to less degree, becomes more apparent in the resting phases. The figures accompanying this account show quite clearly that the nucleolus of Solanum is derived from slightly alveolized portions of chromosomes, and does not arise suddenly and from an unknown source in the late telophase, as Martins-Mano (1905) concludes. Lutman (1925) also seems to assume that the nucleolus does not have a chromosomal origin. This erroneous conception can very well be understood upon examination of their illustrations. They apparently skip too many early stages and consequently overlook the repeated coalescence of large chromatin bodies which results in the appearance of the nucleolus. Although the chromosomal origin of the nucleolus is evident in Solanum it is not nearly so clear as in Phaseolus. In the latter the distal portion of the chromosomes do not become alveolized but round off to form the nucleolus (Kater, 1926, figs. 4–8). The development of this body in the potato is more similar to the condition in the frog and rat than to that of the bean.

The Interkinetic Nucleus.—The distinction between interphase and resting nuclei is a rather arbitrary one. It is also making differentiation and perhaps senescence a part of the definition of 'rest'. However, the terms are valuable providing their exact connotation is understood. Interphase, then, refers to the interkinetic period in rapidly dividing cells, such as the promeristem of a root-tip. Resting nuclei, on the other hand, are from cells that are dividing slowly and which consequently are somewhat differentiated or even senescent. The term for intermediate forms would be a matter of choice.

The interphase nucleus of Solanum contains one or more large nucleoli, the size being inversely related to the number. Relatively large achromatic globules are frequently found suspended in the chromatin substratum of the nucleolus (fig. 10, Pl. 4). The vertical walls of the chromosomal vesicles are again visible where they traverse the wide, hyaline perinucleolar zone. Sometimes they can be traced through the reticulum to the nuclear membrane, but in the majority of cases the reticulum, which is more darkly stained than they, obscures them. The sheaths of the vesicles give the impression of a mechanism for suspending the nucleolus in the midst of the perinucleolar zone. This hyaline zone is sharply delimited from the reticulum which occupies the rest of the space out to the nuclear membrane.

The disperse chromatin (reticulum) presents an aspect quite different from the condition described above for the late telophase. The 'granules' are much smaller and are in continuity with each other, the chromatin again being the continuous phase. Achromatic spherules are suspended in its meshes. Within the continuous chromatic element one can discern more densely basophilic granules of variable size. Some of these are relatively large (fig. 10, Pl. 4).

In a resting nucleus, such as those found in the zone of maturation of a root, the nucleolus is much smaller than in interphase and has to a great extent lost its affinity for basic dyes. Occasionally it is visibly, though finely, vacuolated, which is probably connected with the loss of basophilia. The perinucleolar zone is less extensive than in interphase, and the reticulum suspends much larger globules of achromatin and is itself much more homogeneous. The linin sheaths of the vesicles are visible in the hyaline perinucleolar zone and occasionally in the midst of the reticulum (fig. 11, Pl. 4).

The most important feature of the late telophase and interkinetic period as described above is the persistence of the chromosomal sheath in an apparently unaltered condition from the time that it first becomes microscopically visible in conjunction with alveolization of the chromosomes until the nucleus has reached not only a resting condition but a somewhat senescent one (fig. 11, Pl. 4). In addition it has been shown that the vesicles are contiguous, and are not separated by achromatic channels.

The difference in the reticulum of interphase and resting nuclei may be due to a greater degree of physical dispersion in the latter, but it seems probable that the early onset of senescence has actually decreased the amount of chromatin present. Lutman (1925) has shown that in distinctly senescent cells of the potato there is a great loss of chromatin, as compared to meristematic ones. The same phenomenon is well shown by fatigue in the cells of animals (Dolley, 1913, 1925).

It may be well to anticipate the suggestion that if the ‘ strands ’ were sheaths bounding chromosomal vesicles they would not appear so strand-like. This is not true for the same reason that the nuclear membrane is hardly discernible in surface view while in optical cross-section it is very apparent. Likewise the curved surfaces of the vesicle sheath that are nearest to vertical are the most evident. Refraction, of course, plays a part in this.

The Prophase.—The very early prophase varies to some extent depending upon the condition of the nucleus during the preceding interkinesis. When an interphase nucleus enters the prophase the disperse chromatin becomes collected into relatively few granules of appreciable size. This invariably exposes the linin sheaths of the vesicles so that they can be seen throughout their extent from the nucleolus to the nuclear membrane (fig. 12, Pl. 4). At this early period the nucleolus is still quite chromatic. In resting nuclei that are entering division the same processes occur, but, naturally, the picture presented differs in the same manner as the resting antecedent, the chromatin granules being extended over almost the entire nucleus and the linin not being quite so evident. The nucleolus soon loses its basophilia, its chromatin content apparently being drawn off to give rise to more densely basophilic chromatin granules. These granules increase in number and size until the nucleus is fairly filled with them (fig. 13, Pl. 4). Naturally this procedure more or less obscures the linin.

An additional word might be said concerning reversal of phases in the nucleus. It will be recalled that it was not decided above whether the seeming reversal in the telophase is actually correct or is an artifact. In the early prophase when the reticulum disappears and the chromatin takes the form of granules, discrete or connected to form a beaded chain, we are justified in making a little more definite interpretation. Considering the fact that a little later all of the chromatin will be aggregated in a few chromosomes it would seem that this is most surely a true reversal of phases, the chromatin becoming the discontinuous or disperse phase.

The granular effect is soon lost and the chromatin becomes collected in more or less ribbon-like bands (fig. 15, Pl. 4). The bands seem to be located along one side of the vesicles, hiding the linin strands. Like the chromosomes, however, the ribbons are short, frequently not reaching the nucleolus, and the linin can be seen extending inward from their ends. It is especially noteworthy that these bands are single when they first arise. This would discredit the theories of anaphasic and telophasic duality. The nucleolus still remains as a very slightly chromatic body. Shortly after the stage represented in fig. 15, Pl. 4, the splitting of the chromosomes becomes evident.

Figs. 16 and 17, Pl. 4, are two sections of a single cell. These are very illuminating figures. It is to be noted in each that the chromosomes are double and that the sheaths of their vesicles are still intact and can be seen stretching about through the interior of the nucleus. A strand never runs to the dividing point between two daughter chromosomes. If there were such a thing as a true duplication of the chromosomes in the telophase they would have to be separated by a linin partition, in the light of the present work, and we would expect to find linin sheaths separating them in figures such as those under discussion. The linin sheaths in these figures also show that the vesicles maintain their integrity even after the chromosomes are formed. In fact they are still visible in some areas after the nuclear membrane has started to disappear (fig. 18, Pl. 4). The nuclear membrane and the internal linin eventually dissolve and the contents of the nucleus and cytoplasm become mixed.

How does the new linin sheath of the chromosomes arise ? Can continuity be applied to it also ? These questions cannot be answered. The present condition is very similar to that found in the rat (Kater, 1928) and in that case it was suggested that the new sheath might be formed from one side of the old one. The fact that the prophase chromosomes always appear along one side of their vesicle might be interpreted to support such a suggestion. The nature of this problem, however, will make it very hard to solve. It is perhaps impossible to do so on the basis of morphological study alone. It may be, of course, that the new linin is differentiated anew from the surface of the chromatin as a precipitation membrane, but one is ordinarily loathe to suggest de novo origin unless there is something to support it.

In Solanum there is always a vestige of the nucleolus remaining after the chromosomes are formed. During the process of the development of the latter one finds a progressive decrease of the basophilic character of the nucleolus. This gradual transition is illustrated in figs. 12–18, Pl. 4, beginning with a chromatin nucleolus and ending with an acidophilic plasmosome. After the linin has dissolved and the chromosomes are lined up on the equator the nucleolus takes a dumbbell shape and also extends across the equator (fig. 19, Pl. 4). It very quickly disappears.

Summary of Observations on Solanum.—(1) The numerous small chromosomes come together at the poles to form a densely basophilic mass in which the individual chromosomes cannot be distinguished. (2) The early alveolization of this mass shows that the nuclear membrane is derived from its border and that there are no interchromosomal channels formed. (3) Extension of alveolization discloses the linin sheaths of the vesicles which extend inward from the nuclear membrane. (4) The nucleolus arises from non-alveolized portions of chromosomes. This body gradually becomes achromatized and a vestige of it remains until the next prophase. (5) The chromosomal vesicles maintain their integrity during interkinesis and well through the next prophase. (6) The present interpretations would discredit the theory of telophasic duality.

LYCOPERSICON ESCULENTUM.

Mitotic Processes compared with those of Solanum.—The chromosomes of Lycopersicon are of medium length and not so numerous as in Solanum tuberosum. In their migration to the poles they typically assume a U-shape (fig. 20, Pl. 4). When they become contiguous at the poles of the spindle these U's are frequently compressed, having the two arms of the U pressed in upon one another. Consequently some of the chromosomes form two bands across the 'tassement polaire'. Their arrangement at the poles is far from regular, one arm of a U occasionally extending out into the cytoplasm in a manner similar to the 'finger-like' processes which Boveri has described in the reconstituting nuclei of Ascaris megalocephala (fig. 21, Pl. 4). Such processes in the daughter nuclei of the tomato are admirably suited to a study of the reconstitutional changes. We find that their alveolization results in the formation of a vesicle as described above for the potato (fig. 22, Pl. 4). Very few illustrations have been taken from the tomato. For the sake of economy they have been kept at a minimum, only enough being introduced to show that the conditions are fundamentally the same as in the potato. Fig. 22, Pl. 4, represents about the same stage of division as fig. 5, Pl. 4. It is to be noted that in the former there is more inclination to restrict the early alveolization to the proximal side of the chromosome mass. In this respect Lycopersicon resembles Phaseolus (Kater, 1926, fig. 4). In fig. 22, Pl. 4, the bands separating the alveolized areas are quite chromatic. However, continued imbibition of achromatin converts the chromatin on the bands into the developing reticulum and leaves achromatic linin sheaths in exactly the same manner as in the potato, resulting in figures comparable to 6.

The nucleolus arises from non-alveolized portions of chromosomes and in the late telophase and interphase is very basophilic (fig. 23, Pl. 4). The apparent reversal of phases is again found. The disperse chromatin of the late telophase appearing as discrete granules scattered about in the vesicles. The latter radiate from the nucleolus as before.

The interphase nucleus of Lycopersicon esculentum is identical with that of Solanum tuberosum except that the perinucleolar zone is not nearly so large (fig. 24, Pl. 5). With a picture in mind of the hyaline perinucleolar zone as it appears in the interphase of both the potato and tomato we might consider whether or not it is an artifact. The condition in the onion is quite different and will be dealt with below. Many are inclined to consider this an artifact (Sharp, 1913; de Smet, 1914). However, in the two plants under discussion it seems to me that such is probably not the case. In the first place the perinucleolar zone is always present in interphase nuclei, but not invariably so in resting nuclei. In the latter state the reticulum is probably normally attached to the nucleolus. In addition to being an invariable accompaniment of interphase the area outside this hyaline zone presents a very normal alveolar structure. If the so-called reticulum had contracted from the nucleolus we would expect the achromatic globules of the disperse phase to be tangentially flattened. Such is not the case. We might emphasize here the fact that the interkinetic nuclei of both the tomato and potato are not reticulate as in some plants and animals, but foam-like.

The prophase presents no differences from the account given for Solanum tuberosum except that the nucleolus is a little more ephemeral, usually vanishing before the nuclear membrane is entirely gone (figs. 25, 26, and 27, Pl. 5). In fig. 27, Pl. 5, two chromosomes are embedded in the remains of the nucleolus, as if they had been formed there.

ALLIUM CEPA.

Mitotic processes have probably been followed by the human eye more frequently in the root-tip of Allium than in any somatic tissue of other organisms. Therefore it might seem presumptuous for one to undertake the study of such material with the hope of elucidating problems of cell structure. However, the general lack of agreement on fundamental questions relating to nuclear reconstruction makes any organism a legitimate field for investigation. Perhaps there is more opportunity for correlating the previous and current ideas from material which has played a part in the controversies.

Reconstruction of Daughter Nuclei.—It is a matter of common knowledge that the chromosomes of Allium are relatively very long and thick, and that in their migration to the poles they typically assume a U- or V-shape with the bottoms of the V's towards the pole to which they are travelling (fig. 28, Pl. 5). As a consequence of this, when they become enmassed at the poles (' tassement polaire ') each one is folded back upon itself, pushing the arms of the V's together. Thus each chromosome gives rise to two bands across the ' tassement polaire ', parallel with each other and with the long axis of the spindle. During this procedure of bending the chromosomes back upon themselves they are more or less contiguous, some, however, being occasionally separated from the rest (fig. 29, Pl. 5).

In large chromosomes such as those of Allium one can discern appreciable alveolization in the early anaphase. The chromosomes lose the homogeneous appearance which characterizes them at metaphase and exhibit small achromatic spherules suspended in their chromatin substratum (fig. 28, Pl. 5). The imbibition of these achromatic globules continues as the migration proceeds, so that when the ' tassement polaire ' has been formed the globules are quite large and numerous (fig. 29, Pl. 5). The condensation of the chromosomes draws them so closely together that individuals cannot be distinguished except where their ends project freely from the general mass. This packing of the chromosomes so transposes them upon one another that the achromatic spherules pass out of view. By far the greater bulk of the chromosomes is yet basophilic, and such an obstruction of the view of small achromatic globules is to be expected. We have, however, every reason to suppose that they are still present throughout the mass. The individual ends of chromosomes that can be seen show even more alveolization than in preceding stages (fig. 30, Pl. 5). This condition quickly changes. The solid mass of chromatin becomes broken up into alternating light and dark bands (fig. 31, Pl. 5). The figure referred to is almost identical with a photomicrograph of a reconstituting nucleus in Trillium as published by Gregoire and Wygaerts (1904). It is on just this type of picture that the Gregoirian school bases their theory of the ' loosening up ' of the chromosomes. The appearance of alternate chromatic and achromatic bands in the reconstituting nucleus must be explained, and they do so by assuming that the ends of the chromosomes, forming nuclear membrane, remain in contact while their inner portions draw away from each other permitting cytoplasmic material, probably spindle substance, to rush in between the chromosomes. The formation of the reticulum is explained by supposed branching and anastomosing of chromosomes by way of bridging these cytoplasmic intranuclear channels. According to this conception the achromatin of the resting nucleus would be both interchromosomal and intrachromosomal. This theory served its purpose at the time when no better explanation was suggested, but since there is absolutely nothing to support it, it is high time for it to give way to a conception based on observation of more favourable material. There is nothing in the discernible behaviour of the reconstituting nuclei of Allium that will absolutely disprove the above assumption, but there is a great deal that argues against it. In the first place, the ends of the chromatic bands are frequently farther separated than the inner part (fig. 31, Pl. 5). If the nuclear membrane is derived from the chromosomes, as is widely believed, including Gregoire's school (Robyns, 1924), it would be hard to understand this condition. Secondly, the channels are never entirely achromatic. The strongest argument against this ' loosening up ' idea will be shown in the description of the prophase.

Allium does not afford the best of material for investigation of nuclear reconstruction, but, as outlined above, the few discernible steps in the process indicate that the conditions are the same as in more favourable forms, such as Phaseolus and Mus norvegicus. In both of these organisms the vesiculation of contiguous chromosomes is the means of reconstruction (Kater, 1926, 1928). In the former alternating bands are not formed in the vesicles, one end remaining solid chromatin, while in the latter the chromatin and achromatin banding appears exactly as in Allium. In addition the linin sheaths of the vesicles can be seen in this stage of division in the rat. The individual vesicles can be followed on the surface of the nucleus by indentations and extending inward from these points of contiguity of adjacent chromosomes one can see the linin sheaths of the vesicles, in case they traverse one of the achromatic channels (Kater, 1928, fig. 1 *b*). The similarity of the processes in the onion to those found in mitosis in the cell of the rat is sufficient to justify concluding that they are fundamentally the same, that is, the achromatic channels are merely greatly alveolized sections of chromosomal vesicles. However, I am willing to admit that on a basis of my observations on Allium alone I would be at a complete loss for any explanation of the conditions depicted by the accompanying illustrations. Plants with large chromosomes, it seems, afford less opportunity for solving the intricate problems of nuclear structure than those with smaller ones, such as Phaseolus.

The parallel chromatin bands soon have their relationship modified, their distal ends drawing together at one point, forming a relatively large chromatin mass from which the bands radiate. At the same time they become more slender (fig. 32, Pl. 5). The chromatin mass formed from the ends of these bands is the nucleolus. This becomes evident as the bands become entirely alveolized while the central body remains. Linin sheaths occasionally can be seen radiating from the nucleolus also (fig. 33, Pl. 5).

Rarely the chromatin bands seem to be made up of a double row of granules. The appearance which has led to the theories of telophasic duality. Such a seeming duality is unquestionably exhibited by some reconstituting nuclei, but how far we are justified in going with interpretations is another matter. In the middle of the upper nucleus in fig. 33, Pl. 5, a linin sheath extends from the nucleolus out between a row of double granules. This suggests to me that the duplicity is produced by rows of granules happening to be located along the contiguous sides of two vesicles. This would make the two halves of a double row products of different vesicles. The condition illustrated in fig. 33, Pl. 5, is very rare, which would deter one from basing much on it. In addition, the origin of chromosomes in the prophase is antagonistic to the idea of telophasic duality.

As alveolization of the remaining chromatin proceeds the reticulum becomes more complete. By the time the cell plate has extended entirely across the cell the nuclei are practically in the interkinetic condition (fig. 34, Pl. 5).

The formation of the nucleolus is not always as regular as fig. 32 would indicate. In such a case only one is formed, while the more frequent number in the late telophase is two (fig. 34, Pl. 5).

The Interkinetic Nucleus.—The interkinetic nucleus is typical for plants with large chromosomes. Many animal nuclei are also of the same type. In both interphase and rest the chromatin is the continuous phase and presents a reticulate aspect. The reticulum is made up of moderate-sized granules of deeply basophilic chromatin which are connected by thin sheets of oxychromatin. The granules with their connexions form triangular or rectangular figures, quite at variance with the foam-like state of stained nuclei in the potato and tomato. The resting nuclei of young somatic cells of animals are of the same type as the onion nucleus. The heavy reticulum entirely obscures the linin sheaths of the chromosomal vesicles in well-fixed material (figs. 35 and 36, Pl. 5).

According to the current conception of protoplasmic structure the reticulate appearance of the nucleus must be an artifact, while the nucleus of Solanum preserves more nearly its normal condition.

The nucleolus at interphase is distinctly a chromatin nucleolus or karyosome (fig. 35, Pl. 5). As one follows a single row of cells up the root it is observed that as the cells pass into rest the nucleolus becomes less chromatic (fig. 36, Pl. 5). The net-knots of the reticulum also decrease in basophilia, but not to as great an extent. The nucleolus is typically connected to the reticulum, apparently being merely a gigantic net-knot. The conclusion of Sharp (1913) and de Smet (1914) that the perinucleolar zone is an artifact is probably correct in the case of Allium. Perhaps it is true for all plants having similar-sized chromosomes. Cells which give evidence of distortion from fixation have an appreciable hyaline area surrounding the nucleolus (fig. 37, Pl. 5). Whether or not this is an artifact, its occasional incidence is fortunate because it throws light on the structure of the nucleus. The statement was made above that the heavy reticulum obscures the linin sheaths of the chromosomal vesicles. The pronounced visibility of these sheaths in the perinucleolar zone shows that they are present here as well as in the potato, bean, rat, &c., and justifies speaking of them as present entities.

The Prophase.—Although the telophasic conditions in Allium are of themselves not conclusive they are indicative of homology with Solanum, Phaseolus, Rana, Mus, &c. The origin of the chromosomes in the prophase makes the similarity even more striking. When the reticulum breaks up in the early prophase and the chromatin becomes aggregated in masses or cords one can frequently see that these aggregates are limited by vesicle walls (fig. 38, Pl. 5). There is an inclination for the nucleolus to be located at a meeting-point of the vesicles. This is only partly true in the figure just referred to. The aggregations of chromatin become more chromosome-like as the nucleolus becomes less basophilic (fig. 39, Pl. 5). By the time the polar caps have appeared the chromosomes are fully formed and it is to be emphasized that they are not double. The nucleolus has become, at this stage, very acidophilic and the sheaths of the vesicles are still present (fig. 40, Pl. 5).

The splitting of the chromosomes takes place shortly after the polar caps appear and before the nuclear membrane begins to disappear. They are distinctly double in fig. 41, Pl. 5.

Summary of Observations on Allium.—(1) The large chromosomes become enmassed at the poles in such a manner that each one forms two loops across the nucleus, parallel with the long axis of the spindle. (2) Vesiculation of the chromosomes begins in the anaphase. (3) The underlying process is not at all clear in the formation of alternate chromatic and achromatic bands in the nucleus, but the observable facts indicate that it is probably homologous with the condition in Mus norvegicus. (4) The nucleolus arises from non-alveolized portions of the chromosomes and gradually changes from a karyosomic to a plasmosomic state. (5) There is no perinucleolar zone in well-fixed interkinetic nuclei. (6) The linin sheaths of chromosomal vesicles are visible in the perinucleolar zone, when the latter is present, and also become apparent in the prophase. The splitting of the chromosomes occurs in the prophase.

DISCUSSION.

The Chromosomal Cycle.—The anaphasic and telophasic processes in the somatic cells of these three plants apparently correspond identically with those same processes as disclosed in my earlier studies on Phaseolus, Rana, and Mus so far as fundamentals are concerned. The details of the telophasic transformations in Allium are very like the condition in Rana and Mus, while Solanum more nearly resembles Phaseolus. The underlying physical process is the same as in the early cleavages of the egg, namely, imbibition of achromatin, which is probably an osmotic phenomenon. The reason for thinking it an osmotic transfer of material is that the amount taken up seems to be proportional to the amount of cytoplasm present. If part of the cytoplasm of an early blastomere is removed the developing nucleus will be much smaller and more chromatic than its sister nucleus, which can well be interpreted to mean that the amount of achromatin imbibed by the nucleus depends upon the equilibrium point of an osmotic interchange. In the budding of yeast the same number of chromosomes enter the bud as remain in the parent cell yet the resulting nucleus is smaller (Kater, 1927 *b*).

This conception of the vesiculation of contiguous chromosomes does not preclude the possibility of the chromosomes sending out amoeboid processes before the formation of the 'tassement polaire', but it does mean that the chromosomes are not separated by achromatic channels which are bridged by anastomosing projections from the chromosomes. The Gregoirian school has mistaken lateral alveolization of the chromosomes for 'becoming less closely packed' (Sharp, 1926). Although, as shown in the present work, there is nothing in the nature of proof with regard to anything obtainable from the study of Allium, the conditions so closely resemble those found in Mus that homology is justified. Bolles Lee has lately written (1924) on the reconstruction of nuclei in Paris quadrifolia. He considers that the chromosomes of the 'polar clump' ('tassement polaire') are separated in their mid-region by the appearance and growth of achromatic globules between them. In the interphase these large alveoles lose their integrity and their substance becomes disseminated through the nucleus. In the light of the present series of studies it can be safely said that the filaments which he interprets as chromosomes are only non-alveolized bands or axes of the chromosomes. The globules are doubtless present, but are probably inside the linin sheaths of vesicles, and instead of pushing the chromosomes apart are merely distending individual vesicles and consequently the nucleus increases in size and the chromatin bands become spaced farther apart.

The metaphase nucleus, then, consists of a specific number of chromosomes which are linin sacs filled with chromatin, while the telophase or interkinetic nucleus consists of the same number of chromosomal vesicles, each one of which arose from a chromosome by a process of alveolization. In the alveolization the linin sheath is unaltered except in the matter of distension. The chromatin remains continuous and the achromatin constitutes the disperse phase of the nuclear system. Therefore the reticulum is made up of a single substance, chromatin. This conception of the reticulum has been especially advanced by Gregoire and his school. The only advance in this direction that the present studies has made is the discovery that the reticulum of each vesicle forms a unit and is not directly connected to the reticulum of the adjacent vesicles. This is especially evident in the young oocytes of the rat. Animal cytologists have been especially inclined to consider the reticulum as a framework of linin with attached particles of chromatin. Richards (1917), who very credibly succeeded in tracing the chromosomal vesicles through interkinesis in the early blastomeres of Fundulus, thought that the linin sheaths of the vesicles sent branches inward to form the reticulum. The structures which he saw were probably thin sheets of chromatin separating alveoli.

The origin and fate of the nucleolus in the three plants used in this investigation are the same as in the rat. It arises from non-alveolized or only slightly alveolized portions of the chromosomes. As the cell progresses through the resting and prophasic stages the nucleolus gradually becomes achromatized, changing from a karyosome to a plasmosome. This is in harmony with the growing idea that the nucleolus is derived from the chromosomes, whether it be a chromatin nucleolus or a true nucleolus (Wilson, 1925). The work on senescence and fatigue (Dolley, 1913, 1925 ; Lutnam, 1925), as well as that on metabolic activity (Huie, 1897), indicates that the nucleolus is a storehouse for chromatin which is inactive.

The exact origin of the chromosomal sheath is yet obscure. The prophase chromosomes are the endogenous offspring of vesicles, but whether the new sheath is formed from the old vesicle wall or is formed de novo as a precipitation product of the chromatin, or what not, is unknown. This problem is beyond the scope of the present investigation, although it is a very important one and would merit a great deal of consideration should a fruitful mode of attack be devised.

Chromosomal individuality.—Genetic continuity has been the central feature of every problem in cytology. The continuity of the cell as a whole was naturally the first great step in the achievement of the early cytologists. This was followed some two decades later by the conclusion of Flemming and Strasburger regarding the continuity of the nucleus. Rabl (1885) suggested a theory of chromosomal continuity which has been supported in one way or another by almost every cytologist. Most of the evidence that has been advanced is of an indirect nature, such as constancy of number and form of chromosomes, maintenance of polyploid and aneuroploid conditions, hybridization studies, &c. Although this type of evidence is very strong the subject of chromosomal individuality must remain hypothetical so long as it is based on inferential evidence.

It remained for Richards (1917) to bring forward direct evidence for chromosomal continuity. He succeeded in tracing the chromosomal vesicles, with distinct outlines, through interkinesis and found a prophase chromosome appearing in each and every vesicle. In the present series of papers direct evidence for genetic chromosomal continuity has been obtained from the somatic nuclei of the bean, frog, potato, tomato, and onion, and from somatic and germ nuclei of the rat. In addition, the same resting structure which is evident in these forms has been seen in the nuclei of a number of other organisms. It is not only probable that the universal method of nuclear reconstruction in Metazoa and Metaphyta is by chromosome vesiculation, but also that the vesicles maintain their integrity during interkinesis. Chromosomal individuality no longer rests solely on an inferential basis, but now has direct evidence to support it. The chromosome can be elevated to the same position as the cell whose continuity was expressed as a fact by the dictum ‘ omnis cellula e cellula ’.

GENERAL SUMMARY.

1. The reconstruction of daughter nuclei in Solanum tuberosum, Lycopersicon esculentum, and Allium cepa is by the vesiculation of contiguous chromosomes.
2. The integrity of the chromosomal vesicles is maintained during interkinesis, and a prophase chromosome is formed in each vesicle before its walls disappear.
3. The anaphasic and telophasic alveolization consists in the imbibition of achromatic globules which become the disperse phase of the nuclear system.
4. The reticulum is considered to be a single substance, chromatin. The variation in staining reaction is probably due to physical dispersion.
5. A plasmosome is merely an achromatized chromatin nucleolus. The nucleolus is derived from chromosomes.
6. It is suggested that chromosome vesiculation is probably the universal method of nuclear reconstruction, and also that the vesicles probably maintain their integrity throughout interkinesis.

REFERENCES.

Bolles Lee, A. (1924).—“ The chromosomes of Paris quadrifolia and the mechanics of their division ”, ‘ Quart. Journ. Micr. Sci.’, **69**.

Bonnevie, K. (1911).—“ Chromatinriefung in Allium ”, ‘ Arch. f. Zellforsch.’, **6**.

Boveri, Th. (1909).—“ Die Blastomerenkerne von Ascaris megalocephala und die Theorie der Chromosomen individualität ”, ibid., **3**.

Déhorne, A. (1911).—“ Recherches sur la division de la cellule. I. Le duplicime constant du chromosome somatique chez Salamandra masculosa et chez Allium cepa ”, ibid., **6**.

Dolley, David H. (1913).—“ The Morphology of functional activity in the ganglion cells of crayfish, Cambarus virilis ”, ibid., **9**.

—— (1925).—“ Morphology of pancreatic cell function ”, ‘ Amer. Journ. Anat.’, **35**.

Gregoire, V. (1906).—" La structure de l'élément chromosomique au repos et en division dans les cellules végétales (Racines d'Allium) ", ' La Cellule ', 23.

Gregoire, V., and Wygaerts (1904).—" La reconstitution du noyau et la formation des chromosomes dans les cinèses somatiques ", ibid., 21.

Huie, L. (1897).—" Changes in the cell-organs of Drosera produced by feeding with egg-albumen ", ' Quart. Journ. Micr. Sci.', 39.

Kater, J. McA. (1926).—" Chromosomal vesicles and the structure of the resting nucleus in Phaseolus ", ' Biol. Bull.', 51.

—— (1927 *a*).—" Nuclear structure in active and hibernating frogs ", ' Zeitsch. f. Zellforsch. u. Mikrosk. Anat.', 5.

—— (1927 *b*).—" Cytology of Saccharomyces cerevisiae, with especial reference to nuclear division ", ' Biol. Bull.', 52.

—— (1927 *c*).—" A cytological study of dormancy in the seed of Phaseolus vulgaris ", ' Annals of Botany ', 41.

—— (1928).—" Nuclear structure and chromosomal individuality. Somatic and germ nuclei of the rat ", ' Zeitschr. f. Zellforsch. u. Mikrosk. Anat.', 6.

de Litardière, R. (1921).—" Recherches sur l'élément chromosomique ", ' La Cellule ', 31.

Lutman, B. F. (1925).—" Senescence and rejuvenescence in the cells of the potato plant ", ' Bulletin 252 Vermont Agric. Exp. Station '.

Martins-Mano, Th. (1905).—" Nucléole et chromosomes dans le méristéme radiculaire de Solanum tuberosum et Phaseolus vulgaris ", ' La Cellule ', 22.

Merriman, M. L. (1904).—" Vegetative cell-division in Allium ", ' Bot. Gaz.', 37.

Moenkhaus, W. J. (1904).—" The development of the hybrids between Fundulus heteroclitus and Menidia notata, with especial reference to the behaviour of the maternal and paternal chromatin ", ' Amer. Journ. Anat.', 3.

Rabl, C. (1885).—" Über Zellteilung ", Gegenbaur's ' Morph. Jahrb.', 10.

Reed, T. (1914).—" The Nature of the double spireme in Allium cepa ", 'Ann. Bot.', 28.

Richards, A. (1917).—" The history of the chromosomal vesicles in Fundulus and the theory of genetic continuity of chromosomes ", ' Biol. Bull.', 32.

Robyns, W. (1924).—" Le fuseau de caryocinèse et le fuseau de cytokinèse dans les divisions somatiques des phanérogames ", ' La Cellule ', 34.

Rosenberg, O. (1904).—" Über die Individualität der Chromosomen im Pflanzenreich ", ' Flora ', 93.

Sarbadhikari, P. C. (1927).—" Cytology of Osmunda and Doodia. II. On the gametophyte and Postmeiotic mitoses in the gametophytic tissue of Doodia ", ' Ann. of Bot.', 41.

Schustow, L. (1913).—" Ueber Kernteilung in der Wurzelspitze von Allium cepa ", ' Anat. Ang.', **43**.

Sharp, L. W. (1913).—" Somatic Chromosomes in Vicia ", ' La Cellule ', **29**.

—— (1920).—" Somatic chromosomes in Tradescantia ", ' Amer. Journ. of Bot.', **7**.

—— (1926).—' Introduction of Cytology.' McGraw-Hill Book Co., New York.

de Smet, E. (1914).—" Chromosomes, prochromosomes et nucléole dans quelques Dicotylées ", ' La Cellule ', **29**.

Sutton, W. S. (1900).—" The spermatogonial divisions in Brachystola magna ", ' Kansas Univ. Quarterly ', **9**.

Vejdovsky, Z. (1911).—" Zur Problem der Verebungsträger ", ' Böhm. Gesell. Wiss. Prag.'

Wager, H. (1904).—" The nucleolus and nuclear divisions in the root apex of Phaseolus ", ' Ann. Bot.', **18**.

Wenrich, D. H. (1916).—" The spermatogenesis of Phrynotettix magnus, with special reference to synapsis and the individuality of the chromosomes ", ' Bull. Mus. Comp. Zool. Harvard ', **60**.

Wilson, E. B. (1925).—' The Cell.' Macmillan Co., 1925.

EXPLANATION OF PLATES 4 AND 5.

All figures drawn with aid of Abbe camera lucida. Figs. 1–27 inclusive from sections 4 micra thick; figs. 28–41 inclusive from sections 6 micra thick. Figs. 8, 11, and 28–41 from root-tips fixed in Bouin's picro-formol-acetic; remaining ones from material fixed in Flemming's strong. All sections stained with iron-alum haematoxylin and counterstained with light green, eosin, or orange G. Magnification 1,800 ×.

PLATE 4.

Figures 1–19 inclusive are of Solanum tuberosum.

Fig. 1.—Anaphase. The chromosomes are irregularly arranged on the spindle.

Fig. 2.—The chromosomes are becoming contiguous at the poles of the spindle. Note that the chromosomes are larger than in fig. 1.

Fig. 3.—The chromosomes have apparently fused into a single mass, ' tassement polaire '.

Fig. 4.—Slightly alveolized areas are appearing in the proximal ends of the chromosome masses.

Fig. 5.—Extension of alveolization.

Fig. 6.—Alveolization has disclosed the presence of the linin sheaths of the chromosomal vesicles.

Fig. 7.—Linin still evident and portions of chromosomes which will form nucleolus non-alveolized.

Fig. 8.—Portions of chromosomes joining to form nucleolus.

Fig. 9.—Nucleolus complete in both daughter nuclei. Linin sheaths of chromosomal vesicles radiating about the nucleolus.

Fig. 10. Interphase. Note the moderately chromatic nucleolus and the linin sheaths of chromosomal vesicles. Chromatin forms the continuous phase of the nuclear system.

Fig. 11.—Resting cell. Note the general decrease of nuclear chromaticity as compared to fig. 10.

Figs. 12 and 13.—Early prophase. The chromatin is becoming collected in large granules. The integrity of the vesicles still maintained.

Fig. 14.—Illustrating the peculiar effect that can be produced in sections, the granules are probably cross-sections of chromosomes.

Fig. 15.—The chromosomes are completely formed and are single.

Figs. 16 and 17.—Two sections of a single cell. The polar caps are forming and the chromosomes are divided. The linin sheaths of vesicles are still present and in no case do they separate daughter chromosomes.

Fig. 18.—The spindle is formed, the nuclear membrane is partly gone, and the nucleolus is very achromatic. Some of the double chromosomes are still enclosed in vesicles.

Fig. 19.—A faint vestige of the nucleolus is forming a dumb-bell across the equator.

Figures 20–3, Lycopersicon esculentum.

Figs. 20–3.—Compare with figs. 1, 3, 5, and 9, to which they are respectively comparable.

PLATE 5.

Figs. 24, 25, 26, and 27, Lycopersicon esculentum. Compare with figs. 10, 13, 15, and 19 respectively.

Figures 28–41, Allium cepa.

Fig. 28.—Anaphase. Note the beginning of alveolization.

Fig. 29.—Most of the chromosomes are contiguous and alveolization more extensive.

Fig. 30.—The 'tassement polaire' of Gregoire. Alveoles visible in projecting ends of chromosomes.

Fig. 31.—The chromosomes are extensively alveolized, laterally, producing the effect of alternate chromatic and achromatic bands.

Fig. 32.—The ends of the chromatin bands are joining to form the nucleolus.

Fig. 33.—Apparent duality. Note the linin sheath of a vesicle separating the two halves of a double row of granules in the upper nucleus.

Fig. 34.—Completion of alveolization and development of the interphasic condition of reticulum.

Fig. 35.—Interphase.

Fig. 36.—Resting cell.

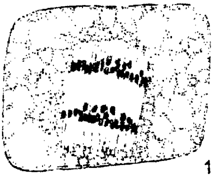
Fig. 37.—Resting condition. Two nucleoli and wide perinucleolar zones. Linin sheaths of vesicles evident in perinucleolar zone.

Fig. 38.—Early prophase. The reticulum has broken up and the chromatin is collecting in strands and masses. Outlines of vesicles prominent.

Fig. 39.—Extension of above process.

Fig. 40.—Polar caps appearing. Chromosomes complete and single. Nucleolus achromatic. Integrity of vesicles maintained. Some chromosomes seen in cross-section.

Fig. 41.—The chromosomes have split. Polar caps pronounced.



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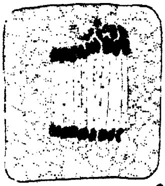

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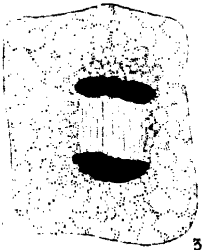

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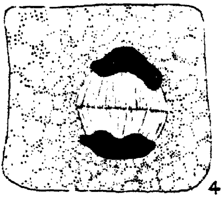

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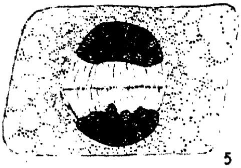

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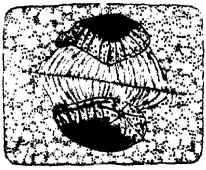

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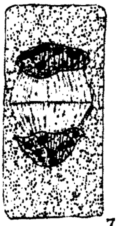

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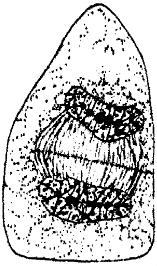

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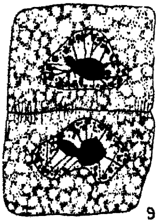

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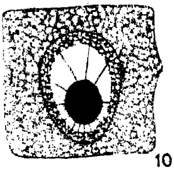

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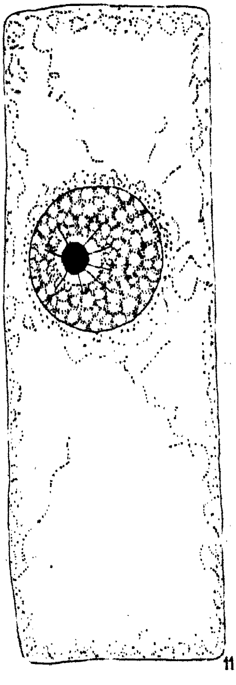

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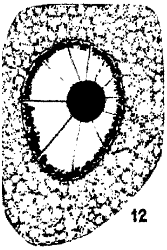
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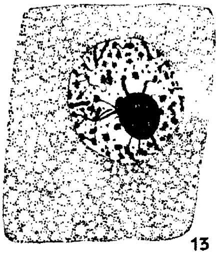
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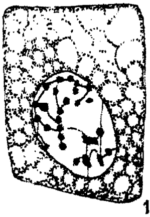
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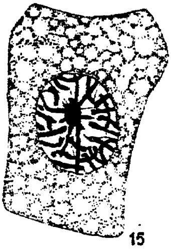
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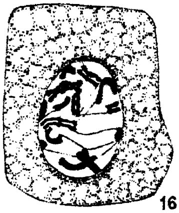
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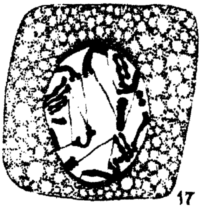
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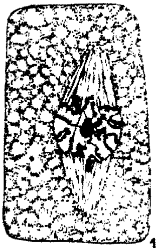
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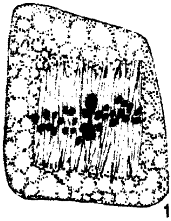
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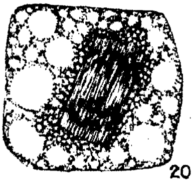
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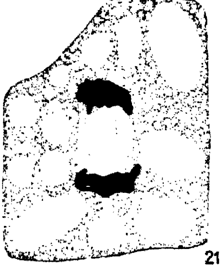
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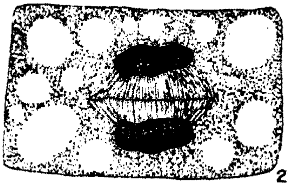
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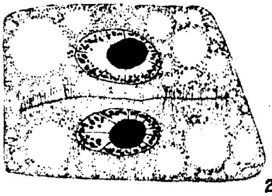
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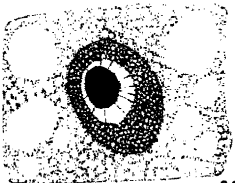

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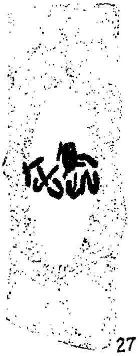

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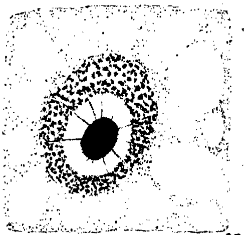

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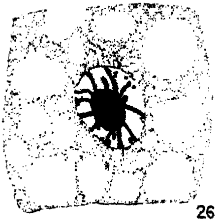

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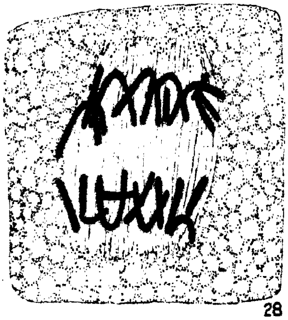

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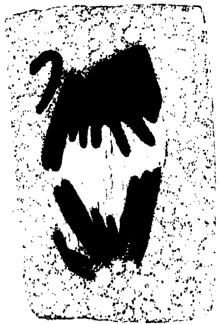

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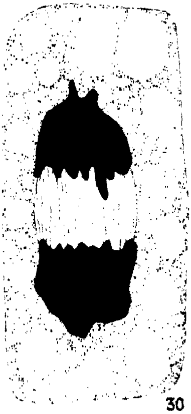

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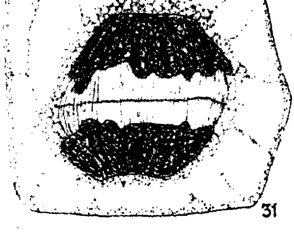

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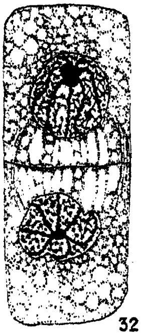

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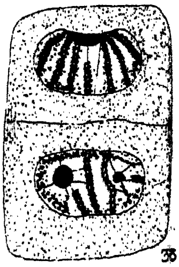

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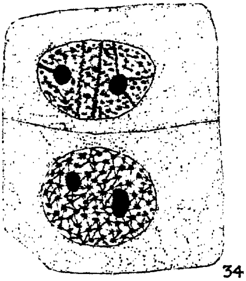

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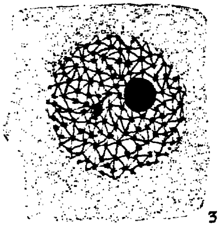

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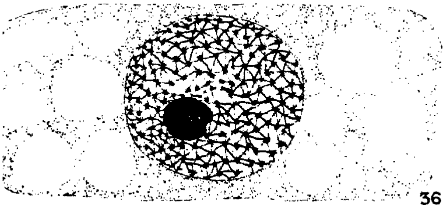

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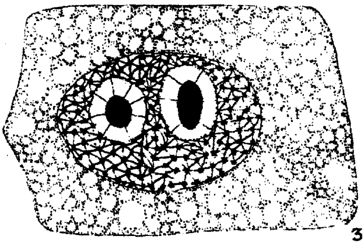

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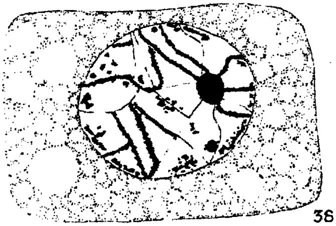

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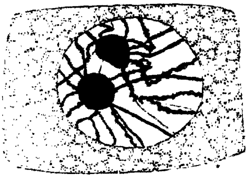

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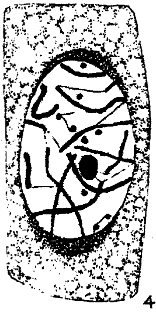

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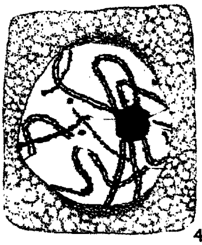


41

Feeding Organs and Feeding Habits of Autolytus Edwarsi St. Joseph.
(Studies on the Syllidae, I)

By

Yô K. Okada, Naba, Hyogo-Ken (Japan).
From the Marine Biological Laboratory, Plymouth.

With 10 Text-figures.

1. INTRODUCTION.

IN connexion with experimental work, especially when an animal is required to be kept alive for a considerable time, it becomes highly important to find out exactly what its food is and how it eats the food. The present work is the outcome of a suggestion made by Dr. E. J. Allen during my residence at Plymouth from June 1927.

A. Malaquin (1893, p. 257) writes in his comprehensive work ‘ sur les Syllidiens ’, that ‘ les aliments arrivent rapidement, grâce aux movements rapides du proventricule, dans l'intestin antérieur. Ces aliments sont de la vase fine, des petits animaux, des invidus appartenant aux colonies sur lesquelles les Syllidiens vivent : Bryozoaires (Bugula, Vesicularia, Membranipora, &c.), Hydraires (Sertularia, Hydralmania, &c.)’. However, he does not indicate what species of Bryozoa or Hydroides Syllids eat.

Syllids at Woods Hole are described as living in a transparent case attached to Hydroids : Autolytus cornutus A. Agassiz associating with Eudendrium and Penaria, Autolytus varians Verrill occurring in abundance on the stems of Parypha, and Autolytus ornatus Verrill among the colonies of Eudendrium and Parypha (P. C. Mensch, 1900, p. 269). But no account has been given of the feeding of these worms. So far as I am aware it is E. J. Allen (1921, p. 132) who has first described it clearly in Procerastea Hallenziana Malaquin.

Procerastea is always associated with Syncoryne: it penetrates with its extruded proboscis the body-walls of the hydroid, just at the base of the hydranth, and pumps out the fluid or semi-fluid substance from the gastral cavity. The proventriculus acts like a pump. According to Allen the rhythm of this movement is at a rate of 150 to 200 per minute.

Autolytus Edwarsi St. Joseph, upon which my observations and experiments have been carried out, is common at Plymouth. Here two forms exist, one of which lives in large numbers amongst colonies of the hydroid Obelia geniculata L., which occurs abundantly on Laminaria. The second form, found in dredgings from the outer ground of Plymouth Sound, is relatively longer and more slender in build (Allen, 1927, p. 874). This form seems to be associated with another species of Obelia (Obelia flabellata Hinck).

Before going farther I wish to take this opportunity of expressing my gratitude to members of the staff of this Laboratory, especially to Dr. E. J. Allen, the director, and Mr. A. J. Smith, the chief laboratory attendant, for their kindness and help.

2. HABITS.

The specimens of Autolytus supplied me seemed to have been living inside a membranous tube, which they had built on Laminaria covered by the colonies of Obelia geniculata L. When the Laminaria with the hydroid was placed in a dish of sea-water the Autolytus left their tubes, and in the course of a few hours crowded at the edge of the water. The worms show distinctly a photo-positive taxis not only to sunlight but also to electric light.

In a glass dish of clean sea-water Autolytus can live for some days without food, and the majority of them form a tube which generally lies horizontally where the water meets the glass, but other tubes are found at the bottom. A few animals wander about the vessel without forming tubes, and still others creep above the level of the water when they dry up and perish.

The tube is a little longer than the actual length of the worm and is open at both ends. It is transparent and, as has been described for Procerastea, sufficiently elastic for the dweller to turn round on itself and travel both up and down the tube. It appears to be woven by the worm itself, with very fine fibrils from a secretion of certain glands in the body-wall.

At Plymouth at least Autolytus is always associated with Obelia, and since Bryozoa and Hydroids are known to be eaten by Syllids, it was probable that the present form lives upon Obelia. This could be most easily tested by giving the hydroid to the hungry worms. I have found that keeping them in a vessel of clean sea-water for two or three days is sufficient to make the worms hungry. With this preparation, when colonies of Obelia were put into the dish, the Autolytus left their tubes almost at once or in the course of a few hours. They first crept along the edge of the water but came down almost directly towards the hydroid. After a short while the animals were seen to creep along the stem of the Obelia and to keep on applying the protruded proboscis to the opening of the hydrotheca. From time to time they stopped at the same spot, and the pumping action, which Allen and Sexton had seen in Procerastea, was clearly observed in the muscular proventriculus. The movement was followed by slow but strong and more or less regularly periodical contractions of the intestine, and a stream of fluid flowed back through the proventricular lumen into the intestine; the animals were evidently feeding. They soon, however, withdrew the protruded pharynx and crept forwards and backwards to apply it to another hydranth. But sometimes they repeated the pumping action upon the same hydranth.

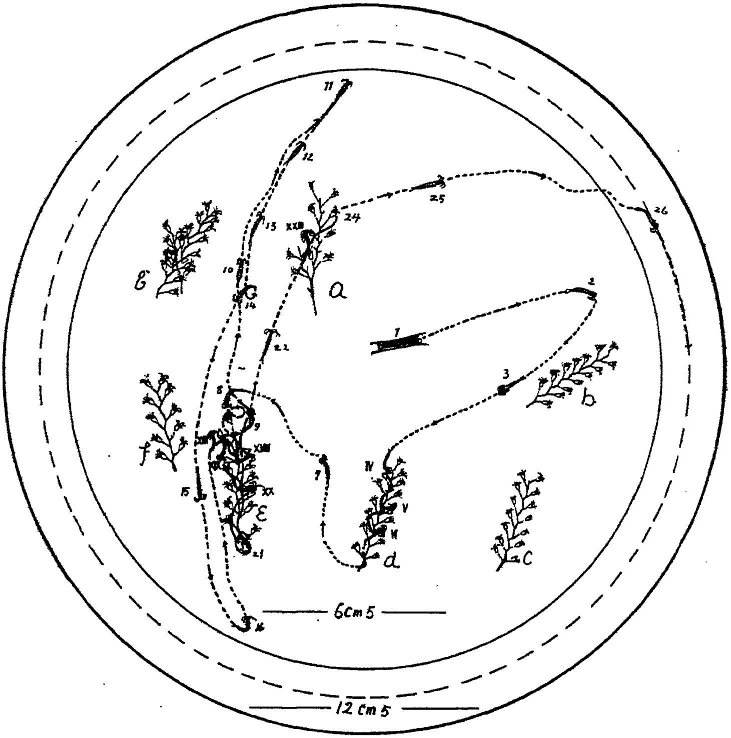
Before entering into the main problem, i.e. the feeding mechanism, I give here with the aid of a diagram (Text-fig. 1) an illustration of the behaviour of a hungry worm when colonies of Obelia were put into the glass dish which contained it.

In this case the experimental animal had built its tube in the middle of the bottom (position 1). As soon as the hydroids were put into the dish the animal left the tube and crept almost straight forwards, until near the edge of the bottom (position 2). Here it turned its head to the right side and, changing direction, crept back to the centre. Passing by one hydroid (*c*) it was attracted by the other (*d*), which was placed more centrally. The Autolytus fed three times on different polyps (*IV*, *V*, and *VI*) and abandoned this colony. It came up to the next hydroid (*e*), but making two complete rotations (at the positions 8 and 9) at the upper extremity of this hydroid, it proceeded forwards without touching any one of the hydranths. Arriving at the edge of the bottom (position 11), the animal seemed to hesitate to proceed farther. Actually it soon came back to position 14 by a backward movement and made a rotation; it then went forward and came down to the opposite side of the bottom of the dish (position 16). It again changed direction, and crept back towards the hydroid (*e*) which it previously arrived at. This time the Autolytus fed upon this colony, beginning with the hydranth of the subterminal branch (*XVII*) and its fellow of the opposite side (*XVIII*). It repeated the characteristic pumping action on a third (*XIX*) and fourth hydranth (*XX*). The well-fed animal came down to the base of the hydroid stem (position 21), then climbed the stem up to the summit and left the hydroid. On reaching colony *a* the Autolytus applied its proboscis twice to hydranths, and once (*XXIII*) was observed to feed for a short while, but soon gave up. Then the animal completely abandoned the hydroids. It proceeded forwards in a hurry to the edge of the bottom and, without stopping there for any time, continued upwards to the edge of the water (position 26): it began its habitual promenade around the glass at the water level. Next morning the animal was found to have formed a fresh tube, lying horizontally at the level where the water meets the glass instead of coming back to its old dwelling.

It is quite clear that Autolytus lives upon the hydranths of Obelia. But such a simple observation as described above still leaves doubt as to whether the worm sucks up the fluid substance from the gastric cavity of the polyp, as in the case of Procerastea, or whether it eats some part of, or the entire hydranth, as the Aeolids do.

Hoping to throw light upon this problem I have made camera

TEXT-FIG. 1.



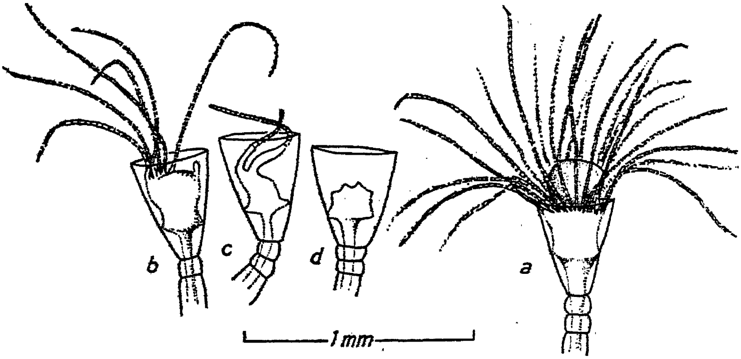
Behaviour of a hungry Autolytus when pieces of Obelia-colonies were put in the glass dish. The different positions of the worm in the course of the observation are indicated by figs. 1–26, the numbers and positions of its feeding being replaced by Roman figures (iv–xx). Small arrows indicate the direction, and the dotted line (in some places replaced by a full line) the course which the worm has followed. The outline of the dish (with a diameter of 12·5 cm.) is shown by a circle in thick line, that of the bottom 6·5 cm.) by a light line, and the edge of the water by a circle in broken line. Observation was continued for about half an hour.

LETTERING COMMON TO ALL TEXT-FIGURES EXCEPT TEXT-FIG. 6.

a, anterior; *ch*, chitin; *co*, buccal cavity; *cp*, peripharyngeal cavity; *cs*, segmental cavity; *dt*, pair of glands opening into proboscis; *dv*, dorsal vein; *e*, eye; *ep*, epithelium; *g*, ganglion; *gc*, cephalic ganglion; *gl*, gland (*l*, ventral; *u*, dorsal); *go*, infra-oesophageal ganglion; *l*, lower; *in*, intestine; *m*(*M*), muscle; *MC*, circular fibres of proventriculus; *ml*, longitudinal fibres; *mm*, motor muscle (*a*, anterior; *p*, posterior); *mp*, parapodial muscle; *MR*, radial columns of proventriculus; *mr*, radial fibres; *ms*, somatic muscle (*l*, ventral; *u*, dorsal); *MV*, ventricular muscle; *n*, nerve; *o*, mouth or oral position; *p*, peritoneal membrane; *pl*, cytoplasm; *prv*, proventriculus; *Q*, quarter of circumference; *R*, raphe (*l*, ventral; *u*, dorsal); *st*, septum (*l*, dorsal; *u*, ventral); *tr*, pharynx; *u*, upper; *v*, ventriculus.

drawings of all the hydranths attacked by the worm. Representative drawings are reproduced in Text-fig. 2. *a* represents a normal hydranth of Obelia geniculata L. with well-extended tentacles. *b*, *c*, and *d* are hydranths of the same hydroid but attacked by Autolytus in different degrees: in *b* the hypostome and most tentacles are missing, in *c* a still greater number of tentacles are wanting, and in *d* only a small basal portion of the hydranth is left. Autolytus apparently eats the tentacles and the upper portion of the hydranth as the Aeolids do, and does not only suck up the gastric fluid of the polyps as Procerastea does.

TEXT-FIG. 2.



Hydranth of **Obelia geniculata** L. (*a*) and some of the same attacked (*b*–*c*) by **Autolytus**.

An actual observation on the feeding animal showed Autolytus attacking the hydranth and eating its tentacles on one side. As will be shown later, the tentacles are drawn into the chitinous tube of the extruded pharynx (proboscis) owing to the negative pressure established there by an effective pumping action of the proventriculus, while the toothed crown (‘ trépan ’) was seen to be used as a saw to cut the tentacles from the hydranth. This action is performed by an upward and downward movement of the head, the protruded pharynx being pushed obliquely into the hydranth cavity and the toothed crown pressing the base of the tentacles against the inner wall of the perisarc (hydrotheca). To cut off all the tentacles from a hydranth requires about 70 to 75 seconds, but most worms abandon a hydranth in from 20 to 30 seconds, before cutting off all the tentacles, and creep up to another individual.

The pumping action of the proventriculus is particularly pronounced at the outset of feeding. It is due to a strong pulsating contraction of the whole organ, the rhythm being at a rate of about twice per second.

TABLE 1. PULSATION OF PROVENTRICULUS.

Duration of the beats.	*Number of Contractions.*	*Rhythm per Second.*
15·3 seconds	20	1·3
17·5 ,,	25	1·3
8 ,,	13	1·6
10·2 ,,	17	1·7
17 ,,	31	1·8
14 ,,	26	1·8
6 ,,	12	2
7·5 ,,	15	2
5 ,,	10	2
10 ,,	21	2·1

Weak contractions may be continued for some seconds longer, or sometimes a spontaneous pulsation is re-established; but in general the pumping action of the proventriculus does not last for the entire duration of feeding; it dies out after a short time, and a strong peristalsis of the intestine appears (this peristalsis is rarely observed in the resting animals).

It is therefore quite certain that Autolytus eats Obelia, but hydranths in sound condition have always been found less liable to attack by the worm. The latter seems to search for the weaker individuals, and the same hydranth was attacked two or three times by the same or different Autolytus.

While creeping up and down the hydroid stem the worms very often touch the dangerous tentacles of the hydroid, which seize the antennae and long cirri of the anterior segments. Nematocysts of the hydroid tentacles are doubtless discharged upon the enemy. The latter, however, shakes its head and quite easily frees itself without any injury.

The gonophores of Obelia are not protected by tentacles, but they are seldom attacked by the worms. In the whole course of my experiments only once was an Autolytus observed to penetrate with its extruded pharynx the cavity of a gonotheca and suck up the medusoid buds.

The gonotheca of Obelia geniculata L. has the shape of a flower-vase with a small pore in the middle of the wider end. Whether or not this pore is large enough to let the pharynx in has not been studied, but the worm will hardly venture to insert its sucking apparatus through this narrow pore while plenty of the edible matter is more easily accessible.

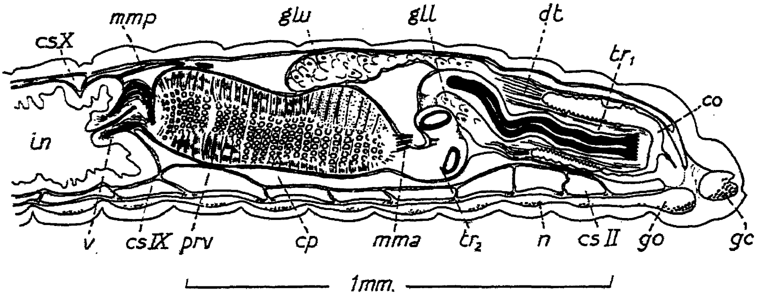
Autolytus Edwarsi St. Joseph lives upon Obelia geniculata L.—Does this hydroid alone provide food for Autolytus or does the worm eat the other species also ? To determine whether or not the animal is monophagous, I put first several colonies of different hydroids including Obelia flabellata Hincks (this is the hydroid with which Autolytus in the dredgings is generally associated), Antennularia antennia L., Sertularia sp., &c., but not Obelia geniculata L., into a glass dish of clean sea-water in which a number of experimental animals had been kept. (The experiment was done with eighteen individuals.) The Autolytus left their tubes as usual and came down towards the colonies of Obelia flabellata Hincks, and fed upon the hydranths just as in the experiment with Obelia geniculata L. They were not, however, attracted by other hydroids. Next, in the same dish which had contained the species of hydroids named, there were put some colonies of Obelia geniculata L., and these were watched at frequent intervals to note the attitude of the experimental animals towards this hydroid when it was present in company with other species. During the first hour they fed five times upon Obelia geniculata L. and twice upon Obelia flabellata Hincks, but not at all upon other hydroids. In the next hour eight times upon the first species of Obelia and three times upon the second, but never upon the others. Lastly, when all the colonies of both species of Obelia were withdrawn from the dish the Autolytus did not come down again to the bottom: they appeared no longer to care about the presence of such hydroids as Antennularia and Sertularia.

It may be mentioned here that Malaquin has recorded the Autolytus in question on the French side of the Channel living amongst Algae (Floridae) covered by Membranipora pilosa L. (loc. cit., p. 306).

3. Feeding Organs (Text-fig. 3).

Although the structure and arrangement of the feeding organs in Autolytus have been dealt with in considerable detail by Malaquin (loc. cit., pp. 187–263), his description is somewhat scattered through the pages of his long monograph. It may therefore be useful to summarize it with such additions as I have been able to make out in the species under consideration. In Text-fig. 3 is a longitudinal section of an Autolytus. In this figure the principal organs of feeding and the general structure of the anterior part of the worm are shown, while the anatomical details of these organs will be found in subsequent figures.

TEXT-FIG. 3.



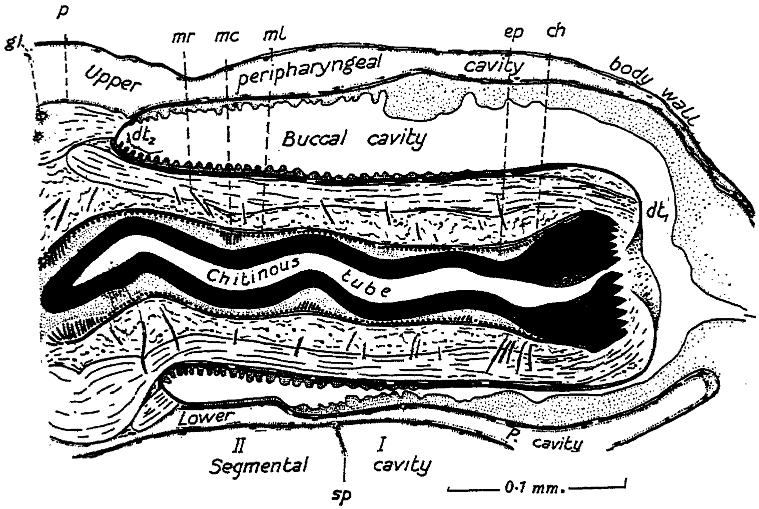
Text-fig. 3 is a median (not in the proventriculus) longitudinal section. Internal division is lacking in the dorsal portion of the body-cavity as far back as the tenth setigerous segment, i.e. the front part of the intestine; the peripharyngeal cavity ('cavité péri-proboscidienne' of Malaquin) is thus continuous (*cp*). In the ventral region, on the contrary, the body-cavity is distinctly divided by transverse septa into as many chambers (*cs*) as there are segments (the tentacular segment has no such cavity). The peripharyngeal cavity contains the principal organs of feeding, which consists from the anterior of (1) the mouth opening to the exterior on the mid-ventral surface of the head; (2) the pharynx (*tr*), a long and sinuate organ with a chitinous tube crowned by a band of small uniform teeth; (3) the proventriculus (*prv*), of an enormous size, with strongly muscular walls; (4) the ventriculus (*v*), which represents a short connecting portion between the pharyngeal part and the intestine; and (5) the intestine (*in*). Besides these there are a large number of unicellular glands which attain a considerable development and consist of two bodies, a large dorsal (*gld*) and a small ventral (*glv*), both stretching from the anterior end of the pharynx to the ventriculus. In Autolytus Edwarsi St. Joseph, as has been described, the glands are not incorporated into the tissue of the pharynx except in the short anterior part (*tr*) of the latter (' région antérieure ' of the French author), which is extruded through the mouth when feeding is going on.

A. Pharynx (Text-fig. 4).

With Malaquin the sinuate pharynx of the Syllids may be divided into three parts. In the present species the ' région antérieure ' alone has the glandular sheath, while the other two regions are without it. So that the pharynx in this case consists of two parts: anterior region or proboscis, with glandular sheath, and posterior region, without it. The first part topographically represents the ' région antérieure ', but structurally corresponds both to the ' régions antérieure et moyenne ' of the other Syllids; the second part topographically to the ' régions moyenne et postérieure ' but structurally to the ' région postérieure '. These differences are, however, due to the different state of development of the pharyngeal glands, and the principal structures of the pharynx remain the same both in the different species of the Syllids, as well as in the different regions of the organ in the one and same species. It consists of an epithelium with a thick chitinous investment, forming the internal tube (Text-fig. 4, *ch*), a muscular coat with internally circular and externally longitudinal fibres (Text-fig. 4, *mc* et *ml*), and a glandular sheath in the anterior region (*dt*) or a very thin peritoneal membrane only in the middle and posterior regions (*p*).

The chitin (*ch*) is almost uniformally thick (7μ) throughout the entire length of the tube, which has an internal diameter of 25–30μ. At the anterior end of the pharynx the tube widens and the chitin thickens, and thus are formed twenty-four small teeth regularly arranged in a row, the 'trépan'. At the posterior end, where the pharynx meets the proventriculus, the tube widens also; but the chitin does not thicken here. There is a peculiar transverse depression at the junction of the tube of the pharynx and the proventricular lumen, and after this point the chitin diminishes suddenly to a thickness of less than 2μ.

TEXT-FIG. 4.



Front part of the feeding apparatus (first region of the pharynx (proboscis) and its sheath).

The epithelium (*ep*) provides no particular interest in the present investigation.

The muscular coat (*m*) is not conspicuous in the pharynx, its thickness not exceeding 2μ in the combined measurement of the outer longitudinal and inner circular fibres.

As above stated, the anterior region of the pharynx or proboscis has a glandular sheath. This consists of a pair of pharyngeal glands, one of which discharges the secretion at the front end of the proboscis (dt_1), while the other pours its contents into the buccal cavity at the base of the pharyngeal sheath (dt_2). There seems, however, no difference between the two glands in the nature of their secretions.

It must be mentioned that in Autolytus, as in other Syllids with a sinuate pharynx, the anterior end of this organ when retracted lies far behind the opening of the mouth, and a wide space (buccal cavity) is formed by the invagination of the body-walls around the anterior part of the alimental tract.

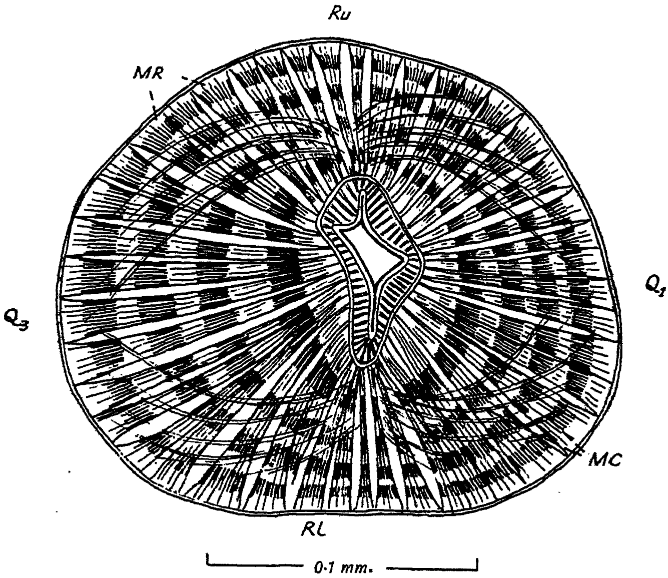
B. Proventriculus (Text-figs. 5 and 7).

The proventriculus is an exceedingly conspicuous and very characteristic structure to which reference is made by all previous authors. It is an organ of the cylindrical shape more than 300μ long. It measures about 120μ in thickness and is strongly muscular. The principal fibres are radially stretched from the centre to the periphery, and this arrangement of the muscle remains the same in every section of any direction, if the plane of the section passes through the centre of the organ. They are visible in the living state with a moderate magnification, even through the body-walls, as small spots arranged in annular bands transverse to the axis of the organ.

The epithelium (*ep*) of the proventriculus (about 8μ) is a little thicker than that of the pharynx, while the chitin is incomparably thin. At both ends, where the proventriculus joins the pharynx as well as where the organ is connected with the ventriculus, a remarkable histological differentiation has occurred in the cellular substance of the epithelium. Malaquin has already noticed (in Autolytus longeferiens St.

Joseph) the peculiar striations in the epithelium near the anterior end of the proventriculus. 'Dans la région antérieure de l'organe, l'épithélium prend un autre aspect, il devient en quelque sorte fibrillaire ; les cellules en sont très allongées, avec noyau médian (Pl. v, fig. 7, *Ep pr*). Cette structure correspond à une disposition particulière, à un épaississement de la cuticule formant en avant du proventricule un aneau chitineux' which is visible in the living state as a 'coupe horizontale' (loc. cit., p. 214). In a more recent publication W. A. Haswell (1921, p. 329) has also stated that the epithelium and cuticle 'both become specially modified towards the anterior end of the organ in connexion with the valvular apparatus' to be described later. Unfortunately both the French and English authors have overlooked the similar modification in the epithelium towards the posterior end of the same organ (see Text-fig. 7). Moreover, the idea of the muscular action of these modified cells does not

TEXT-FIG. 5.



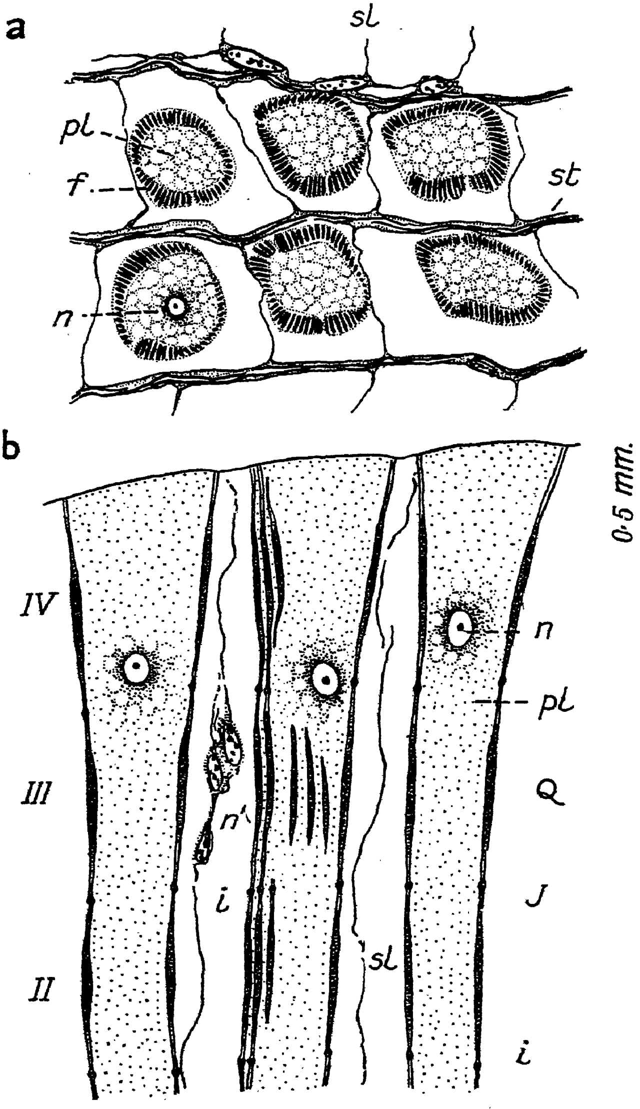
Proventriculus in transverse section. × 360.

seem to have occurred to them; they explain the mechanism of closing and opening in these parts, the function of which is valvular, simply by the construction and relaxation of the radial and annular fibres in the muscle proper of the proventriculus.

The muscular walls are without doubt the seat of the proventricular function, and constitute the principal thickness of the organ. There are two kinds of fibres, one forming the radial columns (*MR*), and the other the slender semi-annular bands (*MC*). Each radial column is a hollow fibre (muscle-bundle or 'Muskelsäule' of Kölliker) with a more or less square cross-section (Text-fig. 6 A), and the large central space is occupied by an undifferentiated protoplasm (sarcoplasm in the more strict sense) with a nucleus near the outer surface. The column is not divided by a transverse septum as described in certain forms, such as Syllis, Eusyllis, &c. A large number of such unit structures are regularly arranged in annular rows from the anterior end of the proventriculus to the posterior.

Each radial column apparently represents a single cell. As regards the contractile elements (the fibrils), these are strictly localized at the periphery of the protoplasm and are differentiated with haematoxylin into stainable and unstainable parts. The stainable part is always longer (12–25 μ) than the unstainable part (8 μ), and the contraction of the fibril seems due to the thickening and shortening of the first part. In the contracted state the stainable part therefore has an elongate fusiform shape with the unstainable part at both ends (see Text-fig. 6 B). According to Malaquin (loc. cit., p. 219) the fibril is composed of alternating zones of ordinary and double refracting material, as in the true striated muscles of the Arthropods and Vertebrates. There are four bands of *Q* (Text-fig. 6) (anisotrophic) in the fibril of Autolytus Edwarsi St. Joseph. Haswell (1921, p. 331) states that the telophragms pass through the bands of *J* (isotropic). So far as my observations are concerned the so-called telophragm seems in this case to be represented by a series of internodular nodules in the middle of the internodes between the contractible zones, the narrow unstainable

Text-fig. 6.



Some radial columns of the proventricular wall. (*a*) In transverse section and (*b*) in longitudinal cut. *f*, muscle-fibres; *i*, internodular nodule; *J*, unstainable part of fibre; *n*, nucleus; *pl*, plasm; *Q*, stainable part of fibre; *sl*, longitudinal septum; *st*, transverse septum. × 550.

part being thickened here a little more than usual. Whether the fibril in a bundle is really connected with the adjoining ones by a transverse septum, i. e. whether the telophragm passes through at the point under consideration, is still quite problematical. As I propose to write an account of the comparative histology of such striated muscles, a description of the 'ino-phragmatic' points is reserved until further studies have been made.

Haswell (loc. cit., Pl. xv, figs. 1 and 2) has figured 'some five or six' nuclei in a single column of Syllis variegata (Grub.) and Malaquin (loc. cit., p. 218) has occasionally found a dinuclear column in Autolytus longeferiens St. Joseph. But these do not represent the usual condition. In general, on the contrary, the unit bundle of the radial fibres of the Syllids is mononuclear. In the present species of Autolytus the nucleus is placed between the third and fourth contractile zones, but more generally near the latter (*n*, Text-fig. 6 B).

The columns of the radial muscle are separated from one another by annular (Text-fig. 6, *st*) and longitudinal septa (*sl*) of a fibrous nature with scattered nuclei. The annular septa are generally thicker than the others, and with Haswell (loc. cit., p. 332) they may be described as containing 'another set of radiating elements'. 'These elements, which for the sake of distinction may be called the accessory or non-striated radial fibres, like the striated, run from the outer fibrous membrane to the inner.' They are placed at regular intervals between the columns of the striated fibres, and their chief function is to provide points d'appui for the annular fibres.

The annular muscles (Text-fig. 5, *MC*) are non-striated. They are more or less compressed in the antero-posterior direction, running for the most part transversely between two adjoining of the radial column, and at the raphes (*Ru* and *Rl*) they seem to be continued straight across the middle line to the opposite side. From the raphes the fibres run in a semi-circularly radial way, and seem to be inserted in the outer membrane near the first (*Q*1) and the third quarter (*Q*3) of the proventricular circumference. According to Haswell (loc. cit., p. 332) these insertions occur between the radial columns, around the corresponding accessory fibres mentioned above. Both Malaquin and Haswell are of the opinion that the annular fibres are constrictors by means of which the lumen of the proventriculus, dilated by the action of the radial muscles, is constricted.

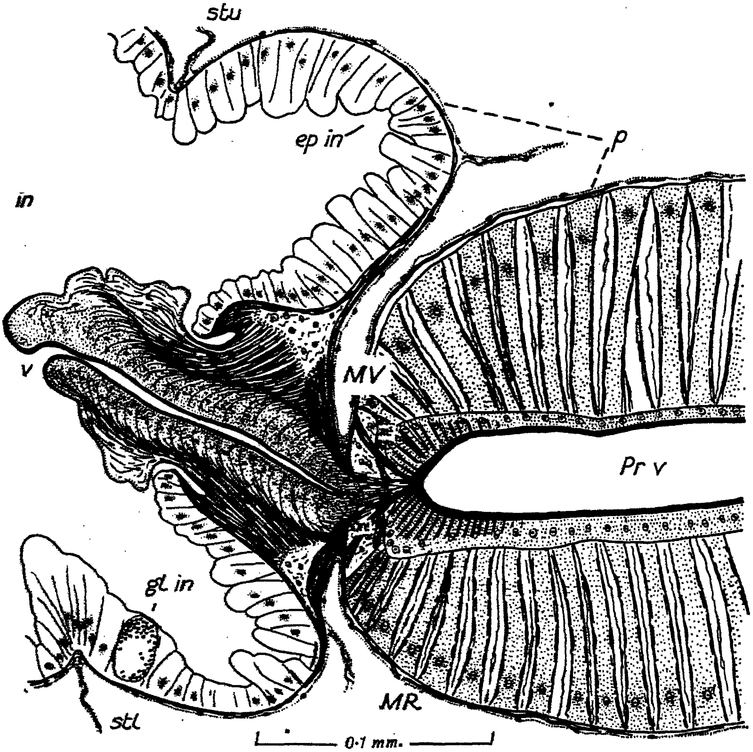
It will be seen, however, from the above description and from Text-fig. 5 that the so-called annular fibres (*MC*) in the present case are so arranged that their function seems to be rather co-operative with the radial fibres (*MR*) than antagonistic to them. In my opinion the constriction—it may be better to say the narrowness of the lumen—is rather the normal condition in the resting stages of the proventriculus, while dilation only requires a strong contraction of the muscles. The diaphragmatic semi-annular fibres seem to bring about some small adjustment of the internal condition of the dilated proventriculus, while the systole of this organ would follow as the natural consequence of the elasticity of the fibres.

I have described the columns of radial muscle as being radially stretched from the centre to the periphery, and that this arrangement of the columns remains the same in every section, whatever the direction of the plane of any section passing through the centre of the organ. But the arrangement shows a little modification in those columns found near the anterior end, where the pharynx is inserted into the proventriculus and the chitin has a transverse groove. At this point the columns, departing from their arrangement in the regular annular zone, run more or less obliquely inwards and forwards or inwards and backwards. The meaning of such an oblique arrangement of the columns will not need further consideration ; it can readily be understood when we consider the valvular action of the anterior end of the proventriculus. What is perhaps a similar modification is also found in the radial columns near the posterior end of the same organ. In the Autolytus under consideration two sets of fibres (one diagonal, Text-fig. 7, m_1, and the other circular (m_2)) are developed around the posterior orifice in addition to the radial columns, and the valvular structure is here more highly specialized than at the anterior end.

C. Ventriculus (Text-fig. 7).

This is a small intermediate chamber between the proventriculus and the intestine. It is greatly reduced in Autolytus. The posterior end of the ventriculus represents the posterior limit of the stomodeal invagination in the embryonic development, and after the differentiation of the larval pharynx this part becomes a small muscular organ with a narrow lumen. The epithelium (about 10μ) is somewhat thicker than that of the proventriculus, but its chitinous investment is thinner. A fibrous metamorphosis has taken place in the epithelium,

TEXT-FIG. 7.



Posterior part of the feeding apparatus (posterior half of the proventriculus, the ventriculus, and the anterior end of the intestine). × 320.

particularly on its outer side; but the original structure—the cells—is still distinctly visible. The cells are neither glandular nor syncytial as described in other Syllids.

There are neither coecae nor post-ventricular parts in this case, the chamber of the ventriculus itself opening directly into the intestine. There is, however, a distinct separation between these two organs, especially in the morphology of their epithelia (see Text-fig. 7).

The outer wall of the ventriculus is strongly muscular; the fibres (*MV*) arise from the posterior part of the proventriculus. They are especially thickened in the ventricular part, but their thickness very soon diminishes posteriorly over the intestinal walls. The anterior part of the intestine, at least, thus has a muscular coat, however thin it may be.

4. MOTOR MUSCLES (Text-fig. 8).

In connexion with the anatomy of the feeding organs, we must now consider the muscles which project and retract the proboscis and move the other parts of the apparatus. Malaquin has already considered this problem, and his description of motor muscles is applicable here with but little change.

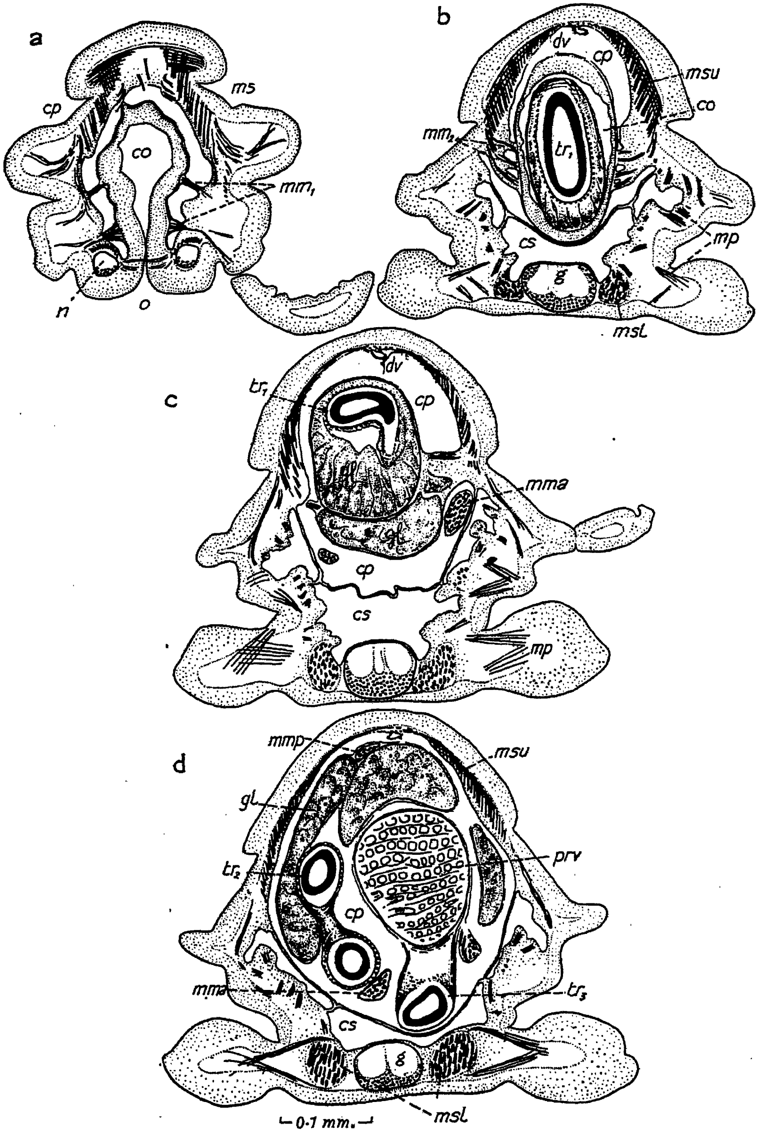
'Tous les muscles proboscidiens s'insèrent, d'une part sur la couche des fibres circulaires de la trompe et d'autre part sur la couche des muscles circulaires des téguments sur une ligne latérale. Ils se divisent naturellement, en muscles protracteurs, chargés de projecter la trompe hors de la bouche et en muscles rétracteurs, chargés de la ramener dans sa situation primitive à l'intérieur du corps. Certains muscles grâce à leur disposition, peuvent jouer à la fois le rôle de protracteurs et de rétracteurs. Il va sans dire que le premier acte étant brusque, rapide, nécessite une musculature plus puissante; tandis que la rétraction de la trompe se faisant plus lentement et nécessitant un effort beaucoup moins considerable que la sortie, s'opère avec une musculature beaucoup moins compliquée' (p. 242).

'La musculature' in the sinuate pharynx 'est surtout condensée vers la première région de la trompe pharyngienne et vers le proventricule (see my figures, Text-fig. 8, which have been drawn from a series of obliquely transverse sections of an Autolytus). La 2^e et la 3^e region de la trompe pharyngienne (corresponding to the part of the pharynx without the glandular sheath) sont complètement dépourvues de muscles protracteurs et rétracteurs. Cela se conçoit facilement, car ces fibres ne pourraient que gêner l'extension des sinuosités de la trompe dans la projection' (p. 244). Malaquin shows several weak muscles at the posterior end of the pharyngeal sheath (' gaîne pharyngienne') where the sheath (' gaîne ') is inserted on the proboscis (' trompe '). (' Les unes, à direction antérieure insèrent sur les parois latérales du corps, les autres beaucoup plus longues vont s'inserer dans le voisinage de la tête. Enfin d'autres à direction postérieure ont un rôle rétracteur.') Besides these I have found fibres (*mm*) of a similar nature near the anterior end of the pharyngeal sheath where the sheath is separate from the body-walls. These muscles have been drawn by Malaquin in Myrianida Pinnigera Montagu (see his fig. 3 in Pl. 4) and also Autolytus longeferiens St. Joseph (see also his fig. 4 in Pl. 4).

As has been demonstrated in many Syllids, there is a pair of strong muscle-bundles on each side of the sinuate pharynx, the function of which may be considered as protractor. These (*mma*) start at the anterior corner of the proventriculus and are inserted on either side of the body-wall near the first segmental septum. I was unable to find in the present species of Autolytus a posterior pair of similar muscles, but a bundle of fibres (*mmp*), as strong as those of the anterior pair, was found stretching between the dorsal wall of the intestine and the pharyngeal glands, which seems to be in some way connected to the dorsal wall of the proventriculus.

Muscle-fibres are also differentiated in the tissue of the septa: these may be to some extent taken into account in connexion with the motor muscles of the feeding apparatus, while the muscles of the body walls (*ms*) and those connected to the movement of the cirri and parapodia (*mp*) need not be specially described here.

TEXT-FIG. 8.



Four sections from the anterior part of an Autolytus cut in an obliquely transverse direction, the upper and lower belonging to three different segments. × 120.

V. MECHANISM OF FEEDING.

That the proventriculus of the Syllids functions as a pump has been recognized by St. Joseph (1886, p. 152). Malaquin (1893, p. 247) has mentioned it also in his work. However, ‘ their descriptions do not suggest ’, states Allen (1921, p. 134), ‘ that they have realized the rapidity of action or the strong current which it can produce ’. Allen records 150 to 200 pulsations per minute in the proventriculus of Procerastea Hallenziana Malaquin. I have counted about two per second, so that there are 120 pulsations per minute in Autolytus Edwarsi St. Joseph. Without any doubt the pumping action of the organ plays an important role in the suction of nourishment through the chitinous tube of the pharynx. ‘Les Syllidiens, en même temps que leurs aliments, avalent toujours une certaine quantité d'eau ’ (Malaquin, loc. cit., p. 247).

The food of Procerastea Hallenziana Malaquin, according to Allen, really consists of semi-fluid matter from the gastral cavity of a hydroid, Syncoryne eximia Allman.

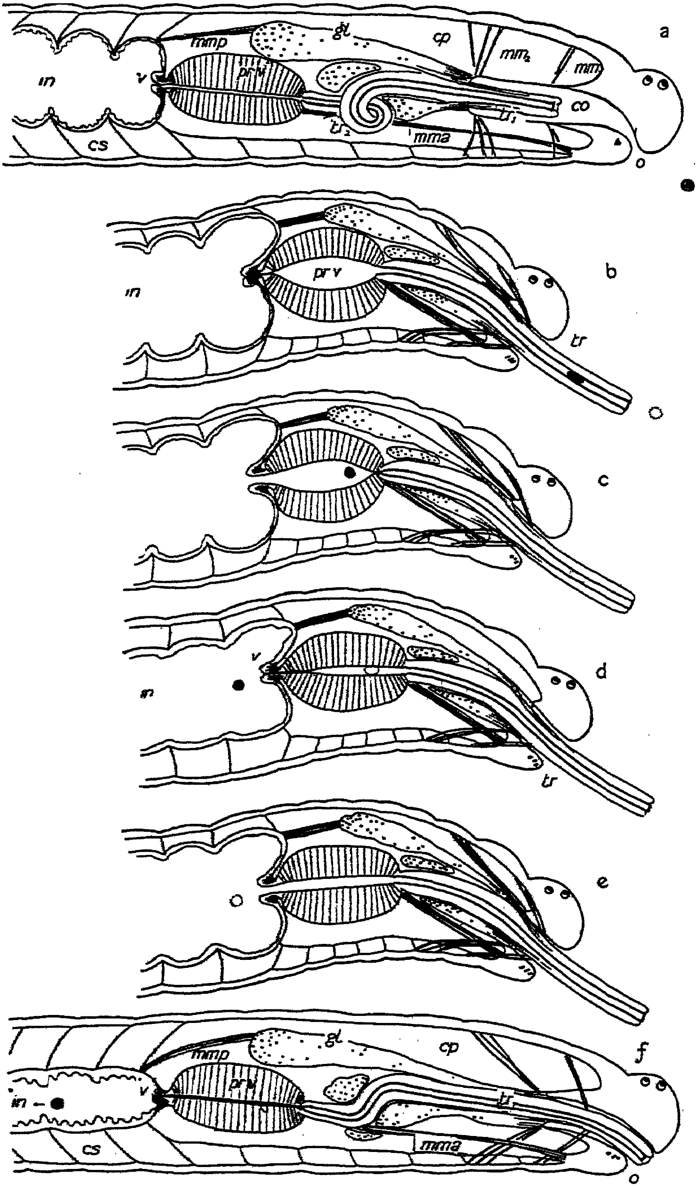
The entire organization of the feeding apparatus, as will be understood from the description of the anatomy of the organs, suggests the system of a suction pump, the pharynx representing the pipe, the proventriculus the pump itself with two valves, one at the anterior and one at the posterior end, and the ventriculus the governor and regulator. But there still remains a question whether the suction is due to an incessant beat of the proventriculus, or whether the pumping movement of this organ is an initial phase of the suction, which is due to the established negative pressure in the anterior part of the alimental tract, i. e. the feeding apparatus. If one observes a feeding Autolytus the pumping movement dies away from the proventriculus in ten to fifteen seconds in most cases, while the sucking action is apparently continued for a longer time. The fluid can be distinctly seen passing through the ventricular lumen into the intestine where it continues in movement. On the other hand a peristaltic movement, with a more or less regular rhythm (about twice per 5 seconds) begins to appear in the anterior part of the intestine at about the same time that the pulsation is disappearing from the proventriculus.

I shall try to illustrate my idea regarding this feeding mechanism by a series of figures (Text-fig. 9) in combination with a graph (Text-fig. 10), as I think it will be better understood in this way than by a long description. Text-fig. 9 A represents an Autolytus in the resting state with the principal organs of feeding shown diagrammatically in longitudinal section. The proboscis (tr_1) is retracted into the buccal cavity (*co*), the posterior two-thirds of the pharynx (tr_2) being folded in a loop. The proventriculus (*prv*) is seen as an ovoid organ (shaded) with a narrow lumen, which opens anteriorly into the chitinous tube and posteriorly into the intestine (*in*) by a short intermediate channel, the ventriculus (*v*). The motor muscles (*mm*) are shown by parallel lines, the body-walls by two lines, and the septa by a single line. An object to be shallowed is indicated by a small sphere obliquely shaded ; it is placed in front of the mouth.

When the worm is going to feed (Text-fig. 9 B) the anterior one-third of the pharynx, i.e. the proboscis, is projected forwards through the mouth opening (*o*). This action is very quick and seems to be achieved by a forward movement of the entire feeding apparatus, including the anterior part of the intestine, due to the strong contraction of the somatic muscles, with the aid of the protractor muscles in front of the proventriculus (*mma*). The sinuate pharynx is straightened and is applied to the object to be eaten. Then the radial muscles of the proventriculus contract with the anterior valve open and the posterior closed. The widened lumen withdraws a certain quantity of fluid from the chitinous tube and the aliment is carried with the stream of water into the tube.

Next (Text-fig. 9 C) the anterior valve is closed and the posterior is opened, the ventriculus being also opened. In this state, when the contracted muscles of the proventriculus relax, all the fluid contained in its lumen will be driven posteriorly into the intestine. By repeating very quickly these actions in

TEXT-FIG. 9

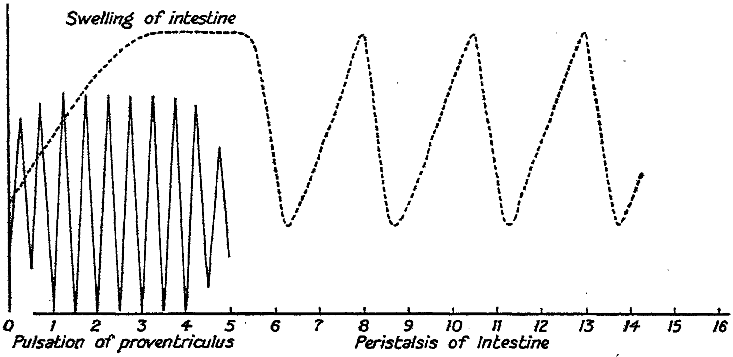


Diagrammatic illustration of the feeding mechanism of Autolytus.

the proventriculus—the action may be called with Allen ‘ pump-action ’ and is represented by a succession of sharp curves in the graph—a negative pressure will be produced in the tube of the pharynx and the food gradually moves back with a current of water.

But when a continuous water-column is established in the front part of the alimental tract the pumping action will no more be needed. The muscular movement now ceases in the proventriculus and the anterior valve remains open, while the water-column is maintained and the suction continued by the peristalsis of the intestine, the posterior valve together with ventriculus now controlling the current. The mechanism of this second period is represented in two figures, Text-fig. 9, *d* and *e*, and the peristaltic movement of the intestine by a large gradual curve in the graph.

TEXT-FIG. 10.



Graphic representation of the feeding mechanism in Autolytus.

Finally (Text-fig. 9, *f*), when the worm stops feeding it retracts the extruded proboscis into the buccal cavity : it extends the contracted segments, draws back the organs of feeding into their proper positions, and the last powerful contraction in the anterior part of the body drives all the food—the hydroid tentacles in this case—farther backwards towards the digestive regions of the epithelium which is characterized by the presence of numerous unicellular glands.

Before closing the description of the present observations, it may be added that the front portion of the intestine at the outset of feeding swells up enormously and overlaps the posterior part of the proventriculus. This phenomenon is particularly clearly seen on the dorsal side.

5. SUMMARY.

Autolytus Edwarsi St. Joseph at Plymouth lives upon Obelia. It deprives the hydroid of the hydranths and eats them.

The worm cuts off the tentacles from the hydranth with the toothed crown of the chitinous tube (the 'trépan'), and sucks them up through the protruded pharynx by establishing in the front part of the alimental tract by the activity of the proventriculus a continuous water-column which is drawn back into the intestine.

The pulsation or pumping action of the proventriculus is particularly strong and distinctly visible at the outset of feeding. After a short time it dies away and the action is followed by peristalsis in the intestine.

The entire organization in the front part of the alimental tract, which is the feeding apparatus of the Autolytus, suggests the system of a suction pump, the pharynx representing the pipe, the proventriculus the pump itself with a valve at each entrance, and the ventriculus the regulator of the water-column.

The proventricular activity is due to the strong muscular development in the radial columns, which are stretched from the centre of the organ to the periphery. In each fibre are four contractile zones, three internodes, and two insertion parts. The contractile zones only are stainable with iron haematoxylin, and these may be comparable to the anisotropic bands of the striated fibre of the Arthropods and Vertebrates. In the middle of the internode there is a thickening, the internodular-nodule: the problem whether this point can be referred to the intrafibrillar part of the inophragm (telophragm in this case) is reserved for future discussion.

REFERENCES.

Agassiz, A. (1862).—" On alternate generation of Annelids and the embryology of Autolytus cornutus ", ' Journ. Nat. Hist. Soc. Boston ', vol. 7, p. 392.

Allen, E. J. (1921).—" Regeneration and Reproduction of the Syllid Procerastea ", ' Phil. Trans. R. Soc. London ', B, vol. 211, p. 131.

—— (1927).—" Fragmentation in the Genus Autolytus and in other Syllids ", ' Journ. Mar. Biol. Assoc.', vol. xiv, p. 869.

Fauvel, P. (1923).—' Faune de France (5. Polychètes errantes) ', Paris.

Haswell, W. A. (1890).—" A Comparative Study of Striated Muscle ", ' Quart. Journ. Micr. Sci.', vol. xxx, p. 31.

—— (1921).—" The Proboscis of the Syllidea (I. Structure) ", ibid., vol. lxv, p. 323.

Malaquin, A. (1893).—" Recherches sur les Syllidiens ", ' Mém. Soc. Sc. Arts, Lille ', p. 1.

Mensch, P. C. (1900).—" Stolonisation in Autolytus varians ", ' Journ. Morph.', vol. 16, p. 269.

Potts, F. A. (1911).—" Methods of Reproduction in the Syllids ", ' Ergeb. u. Fortschr. d. Zoologie ', Bd. iii, Heft 1.

St. Joseph, de (1886).—" Annélides Polychètes des Côtes de Dinard ", ' Ann. Sci. Nat. Zool.', 7, tom. i, p. 127.

Experimental Studies on the Histology of the Mammalian Thymus.

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With Plates 6–12.

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INTRODUCTION.

IT has been known for some time that a variety of experimental methods can be employed to bring about destructive changes in the mammalian thymus, accompanied by a conspicuous decrease in its size. In most cases if the treatment is not too severe (**22**) and the animal young, the organ subsequently regenerates. Injection of serum (**8**), weak acid (**9**), or aniline dyes (**10**), alcohol (**18**), paraffin (**11**), or adrenalin (**12**), fracture of a limb (**13**), and treatment with X-rays (**1, 4, 22, 23**) will all cause partial degeneration of the thymus which may be followed by regeneration. The time which elapses before the organ is completely restored varies with the severity of the treatment, but histological investigation indicates that the process is the same in all cases. Autoplastic transplantation of the thymus into the muscle of the abdominal wall (**14, 16, 17, 21**) is followed by a similar degeneration and regeneration, and pieces of thymus cultivated in plasma show the histological changes that occur in the whole organ during experimental involution.

A section through the normal mammalian thymus is composed of a number of dark cortical areas surrounding paler medullary regions, the two being distinct but not separated by any membrane (fig. 12, Pl. 7). The cortex consists almost entirely of small cells with little cytoplasm lying close together, while the medulla contains similar cells scattered among larger 'reticular' cells with pale nuclei. Blood-vessels ramify throughout the thymus, but are more abundant in the medulla; it is not uncommon to find agglomerations of erythrocytes lying free among the thymus cells (**19**).[1] Conspicuous in the human thymus and in that of the cat, but small in the mouse and certain other mammals, are the nests of more or less concentrically arranged cells known as Hassall's corpuscles, which occur in the medulla (fig. 2, Pl. 6). Strands of connective tissue penetrate the thymus from the surrounding sheath, and may divide it up more or less completely into a number of lobules, each with a distinct cortex and medulla.[2]

The modifications which have been described in the thymus after experimental treatment may be summarized as follows: the small thymus cells in the cortex (lymphocytes of some writers) undergo pycnosis in large numbers, and are rapidly ingested by the larger elements (reticular cells). After about three days the thymus is considerably reduced in size, and most of the nuclear debris have been resorbed; the corpuscles of Hassall are conspicuously enlarged, and in some species ciliated cysts have developed. The outer layer of the thymus consists of cells with large nuclei described as epithelial or reticular. At this stage the medulla contains a number of persistent small thymus cells, and is darker staining than the cortical region—an inversion of the relation in the normal thymus. The blood-vascular and connective-tissue elements have become more prominent, and many polymorphs can be seen in the vessels. Later, the vascular and connective-tissue elements regress, and a rapid proliferation of small thymus cells takes place; after a few days characteristic cortical and medullary regions can once more be distinguished. The corpuscles of Hassall degenerate during this period, and are inconspicuous in the newly reconstituted organ. Histological regeneration may be completed in six days.

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[1] A similar extravasation of erythrocytes has been observed by Dustin (**5**) in the thymus of the axolotl and reptiles, and by the present writer in the Teleost thymus.

[2] A comparison of sections through the thymus of a number of mammalia indicates a connexion between the amount of connective tissue in the normal organ and the size of the corpuscles of Hassall. Thus in the thymus of man, the cat and the marsupial Trichosurus, the corpuscles are large, and the thymus subdivided by numerous strands of connective tissue; in the rabbit, rat, and mouse, on the other hand, connective tissue is relatively scarce in the thymus and the corpuscles insignificant in size.

The discrepancies in the accounts of the writers cited above are largely due to fundamental differences of opinion concerning the histogenesis of the thymus, the nature of the small thymus cells and of the larger elements in the medulla. The controversy extends to the thymus of all vertebrates, and a full account can be found in recent papers by Hammar, Dustin, and Winiwarter **(15, 7, 24)**. It has long been established that the original thymus rudiment is budded off from the epithelium of the pharynx. Hammar and others maintain that the small thymus cells are true lymphocytes, derived from mesodermal immigrants, which have penetrated the thymus during early development and multiplied there; the larger cells in the organ, including the corpuscles of Hassall, are the product of the original epithelium. Amongst recent authors Dustin states, on the contrary, that the small thymus cells are directly descended from the cells of the original rudiment, while the other elements are mesodermal and have passed into the thymus along with the blood-vessels. According to Hammar the thymus consists of lymphocytes lying in an epithelial reticulum; according to Dustin there is no such persistent reticulum, since the original epithelium has been replaced by the small thymus cells to which it gave rise.

The changes in the thymus which degenerates after experimental treatment and subsequently reforms have been studied in detail, partly to throw light on the function of the organ and partly to elucidate its histology. The following summary of the conclusions of different workers with regard to the second problem shows that agreement has not yet been reached on essential details.

Rudberg (**23**) investigated the effect of X-rays on the dog's thymus; he states that lymphocytes—for as such he regards the thymus cells—degenerate, and are ingested by the reticular cells; later these too degenerate. Regeneration is brought about through mitosis of reticular cells or persistent thymus cells, or through immigration of lymphocytes along the connective tissue. The illustrations are inconclusive.

Aubertin and Bordet (**1**) studied the action of X-rays on the thymus of new-born cats and rabbits; they observed that the lymphocytes underwent pycnosis and were replaced by large epithelioid cells, continuous with the cells forming the corpuscles of Hassall; later these epithelioid cells form fibrous tissue. The authors incline to the belief that these are connective-tissue cells. They describe the hypertrophy of Hassall's corpuscles which contain nuclear debris.

The occurrence of this hypertrophy was confirmed by Regaud and Crémieu (**4, 22**) in their more detailed study of the effect of X-rays on the thymus of the cat. They note variations in the small cells (' lymphocytes ') of the normal thymus, and suggest that those in the medulla, which are less sensitive to X-rays, differ from those in the cortex. The regeneration of the small cells is not described in detail. Modifications in the blood-vascular and connective tissue following irradiation are described; a network of fibrous tissue develops in the interlobular clefts and spreads into the organ; the margin of the reduced thymus undergoes sclerosis. Polymorphs increase in number shortly after irradiation, but disintegrate in the corpuscles of Hassall when the organ is regenerating. Regaud and Crémieu are uncertain of the origin of the reticular cells, but believe them to be epithelial; they appear to have a centripetal tendency and to form hypertrophied corpuscles of which the innermost cells are degenerating. The authors comment on the instability of these structures, and the rapidly reversible changes which they undergo in the irradiated thymus.

Dustin (**8, 9, 10**) injected various substances into mice, and studied the effects produced on the thymus. The immediate result of such treatment is the appearance of numerous pycnoses of small thymus cells. An accompanying metaplasia of the vascular and connective tissue takes place, and, from this, corpuscles of Hassall and ciliated cysts develop. Later the persisting small thymus cells undergo mitosis.

Goldner (**12**) found that the injection of adrenalin would bring about degeneration of the thymus. He concludes from his preparations that the reticular cells and corpuscles of Hassall are all epithelial structures. A subsequent study (**13**) of similar changes in the thymus of a guinea-pig, after the fracture of its limb, led this writer to extend his conclusion to the small thymus cells. These too may be changed into epithelial cells, or even into corpuscles of Hassall; they are not lymphocytes.

Jaffe, Plavska, and Gottesmann (**14, 16, 17**) have recently made a detailed study of the degeneration followed by regeneration which takes place in a thymus when the whole lobe or pieces are transplanted into the muscles of the abdominal wall. They assume the existence of a thymus reticulum, and describe all large cells seen in the thymus as epithelial or reticular, the terms being used interchangeably. Little reference is made to the vascular and connective-tissue elements. These writers conclude definitely that the young thymus cells in the regenerated organ together with the corpuscles of Hassall, ciliated 'ducts' and similar elements are all the products of the reticular cells. In this they agree with Goldner and disagree with Hammar and others, since they hold that their evidence shows that the thymus cell is not a lymphocyte.

It is clear from the accounts of the writers cited above that the corpuscles of Hassall increase in size while the thymus cells undergo pycnosis, and degenerate as the thymus cells are being reformed. To assume that these diverse elements are all modifications of the original epithelial tissue raises considerable difficulties, and to substantiate such a theory requires more conclusive evidence than has yet been brought forward.

The most recent paper on the experimental histology of the thymus appears to be that of Popoff (**21**); he studied the changes in small pieces of rabbit thymus grafted into the abdominal wall according to Jaffe's technique, and cultivated in plasma. Popoff refers to the controversy on the nature of the thymus elements, and, unlike Jaffe, concludes in favour of the thymus as a lympho-epithelial organ; the histological changes in the transplant are essentially those found by previous workers, except that no reference is made to the behaviour of the corpuscles of Hassall. The activity of the connective tissue surrounding the graft is described; it penetrates the thymus and resorbs the pycnotic masses. Regeneration is initiated by mitoses in the peripheral layer formed of epithelial cells but, contrary to the statement of Jaffe, it is not these cells that give rise to the small thymus cells; the latter are produced by the connective tissue where it comes in contact with the epithelium —a process described by the author as agreeing with Hammar's lympho-epithelial theory. No illustrations are given.

It is well established (**22, 16, 12, 19**) that the corpuscles of Hassall are by no means constant structures, as was once thought, but highly variable cell aggregations. Often they appear concentric, since cells with large pale nuclei group themselves round the periphery, while at the centre degeneration and liquefaction is taking place. Transitions between corpuscles of Hassall, cysts and normal 'reticular' cells have been traced (**19**). The chief argument from these experiments in favour of the existence of an epithelial reticulum in the thymus (enclosing an infiltration of lymphocytes) is the appearance in the cortex, after the small thymus cells have degenerated, of large epithelioid cells. It is assumed that these are descended from the cells of the original thymus rudiment, and identical with the large cells of the thymus medulla which form corpuscles of

Hassall. It is a matter of dispute whether or not, in the regenerating organ, these epithelioid cells give rise to the small thymus cells; immigration of the latter may be excluded, but their development from embryonic connective-tissue cells accompanying the blood-vessels has been described **(21)**; they do not appear to arise from the small thymus cells which have persisted in the medulla, but are regenerated in the cortex.

It was thought that a further investigation of the histological changes in an X-rayed thymus might clear up certain points which remain doubtful, especially the nature and behaviour of the thymus reticulum. If the reticular cells of the medulla which form corpuscles of Hassall are epithelial, as Hammar maintains, it should be possible to distinguish them from connective-tissue elements by their appearance or behaviour in the X-rayed thymus, and in tissue cultures. In this connexion the origin of the large cells forming a border round the reduced thymus is important. The manner in which cortex and medulla are reconstituted, when the thymus is regenerating, may throw light on the process whereby the normal organ is differentiated from the original epithelial rudiment.

Material and Technique.

The material used was taken from male mice 4–6 weeks old at the time of irradiation, when normal involution of the thymus has not yet begun. Owing to the small size of the thymus in mice compared to other mammals, it is possible to fix and section each lobe with the surrounding tissue in its entirety. This procedure renders the interpretation of sections less liable to error, and reveals any immigration into the irradiated tissue. The irradiation was carried out in the Department of Anatomy, University College, by Mr. F. Melville, under the direction of Dr. A. S. Parkes. The dosage was found to give approximately ¼ B. tint with Levi's pastilles. Details:

Kilovolts, 50.
Milliamps, 5.
Wave-length, 0·285 A.
Spark gap (point-point), 6·3 cm.

Tube, Coolidge universal, medium.
Distance from anti-cathode, 18 in.
Filter, none.
Time of exposure, 80 minutes.

The treatment appeared to have no deleterious effect upon the mice, which were killed at varying intervals after it in order that the changes in the thymus might be followed. The latter was dissected out with its adjacent tissue and fixed in Bouin's fluid; one or both lobes was then embedded and sectioned, usually completely. Sections were cut at 5μ and 10μ, and the following stains were employed among others:

Iron haematoxylin and Van Gieson.
Eosin, iron haematoxylin, and light green (Prenant).
Ehrlich's haematoxylin and Pasini.
Mallory's connective-tissue stain.

THE NORMAL THYMUS IN A 4–6 WEEKS MOUSE.

In a 4–6 weeks mouse no involution of the thymus has taken place. As in all mammals the bulk of the organ consists of the small close-lying thymus cells which have little cytoplasm (fig. 12, Pl. 7); the nuclei of these stain deeply, and vary in diameter from 3·5–6μ (fig. 1, Pl. 6). A few of these cells in the cortex are dividing mitotically, and a few are pycnotic. The medulla is small; corpuscles of Hassall and cysts can be seen in continuity with the pale reticular cells. The corpuscles of Hassall are not large as in the cat, but have a diameter varying from 20 to 40μ. They consist of more or less concentrically arranged groups of cells, of which the middle ones are frequently degenerating (fig. 2, Pl. 6); some of them contain the remains of polymorphs. The cysts are lined by a more or less irregular layer of reticular cells, which are sometimes ciliated (fig. 3, Pl. 6); the larger ones may have a diameter of 60μ and be 90μ long. Their contents include cell debris, leucocytes, and thymus cells. Blood-vessels are most abundant in the medullary areas. Erythrocytes and polymorphs can be found lying free among the thymus cells. The thymus of the mouse (unlike that of the cat, fig. 28, Pl. 11) is not divided up into separate lobules by connective-tissue septa; only thin irregular strands from the enclosing fibrous sheath penetrate the organ.

HISTOLOGICAL CHANGES IN THE X-RAYED THYMUS.

2 Hours after Irradiation.

No reduction in size has taken place in the thymus, but sections show that pycnosis of the small thymus cells has already begun. Groups containing six to twelve pycnotic nuclei are evenly distributed in the cortex, but rare in the medulla. Mitoses are uncommon, and the corpuscles of Hassall normal.

6 Hours after Irradiation.

This thymus is rather smaller than the last, and well-marked histological changes have occurred. The larger blood-vessels have increased considerably in size, and are crammed with red blood-corpuscles; fresh vascular and fibrous tissue is pushing in at the periphery of the organ. The thymus cells in the medulla are almost all intact, but the cortical ones are degenerating in large numbers. It is important to note that mitoses are rare; there are none among the reticular cells of the medulla with pale nuclei. Active amitotic proliferation, however, is taking place in the cortical strands of fibrous connective tissue (figs. 4, 5, 13, Pls. 6 and 7), and a similar activity can be observed in the connective tissue and smooth muscle in the walls of the blood-vessels.[1] Amitosis takes place frequently by simple median cleavage.

The following types of cells can be found in the inpushing vascular and connective tissue: endothelial cells, red blood-corpuscles and polymorphs belonging to the capillaries, and fibroblasts, some with rounded, pale nuclei containing one or more nucleoli, others whose nuclei are irregular or spindle shaped. The first type of fibroblast is indistinguishable from the 'epithelioid' reticular cell of the medulla. Both actively ingest the nuclei of the degenerating thymus cells; the small thymus cells left intact do not appear to be phagocytic (cf. Popoff, **21**). As in the normal thymus, corpuscles of Hassall are comparatively rare, and cysts are few and small.

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[1] See Discussion.

10½ Hours after Irradiation.

At this stage the cortex shows a reduction in size, and is filled with degenerating thymus nuclei (fig. 14, Pl. 7); these are being ingested by connective-tissue cells and polymorphs. The complete disappearance of the outer part of the cortex, and the occurrence of whole areas containing nothing but pycnotic debris shows conclusively that there is no continuous persistent reticulum. Some of the thymus cells in the medulla have degenerated, but the greater number are intact (fig. 16, Pl. 8).

Cysts have become numerous; the diameter of the pluricellular ones varies from 17 to 170 μ. The majority occur in close connexion with vascular tissue, often on the borders of cortical and medullary areas. They are lined by an irregular layer of reticular cells, of which some or all may terminate in a brush of cilia (figs. 6, 7, 15, Pls. 6 and 7). Often the cavities are not round but branching, two or more cysts being connected by diverticula, or by strands of ciliated cells (Fig. 19, Pl. 8). It can be clearly established that the reticular cells lining the cysts are derived from the perithelial connective tissue of the blood-vessels; not only are the staining reactions similar, but actual continuity can be traced[1] (fig. 6, Pl. 6). In many cases cavities have been formed in the thymus through the mass degeneration of the small cells, and have been subsequently lined by the perithelial cells of adjacent blood-vessels. Cysts can be found in process of development which lack a complete border of cells (fig. 6, Pl. 6). Many of the cells lining the cysts are cleaving amitotically; there are no mitoses among these cells, which are frequently arranged in pairs round the border of the cavities, indicating recent cell division (fig. 7, Pl. 6). Among the reticular elements of the medulla in general, mitoses are as rare as in the normal thymus.

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[1] These cysts resemble those found, not uncommonly, in the normal thymus, but irradiation has led to an increase in their number and size. Cf. figs. 3, 6, 7, Pl. 6.

Sometimes the degeneration of a blood-vessel brings about cyst formation; such cysts, bordered by the perithelial cells (which develop conspicuous brushes of cilia), contain degenerating erythrocytes and polymorphs in addition to collagenous debris (fig. 18, Pl. 8).

Cysts of another type may arise through the coalescence of two or more unicellular cysts, which are not uncommon. Others again may be formed by the degeneration of the central mass of epithelioid cells in a corpuscle (fig. 17, Pl. 8). Although the cysts may be formed in various ways they all alike are the product of connective-tissue cells;[1] usually they contain leucocytes and degenerating masses (fig. 15, Pl. 7). It is interesting to note the close similarity between these cysts in the irradiated thymus of the mouse and those described and illustrated by Dustin (**5, 6**) in the thymus of the axolotl and the frog.

Corpuscles of Hassall are more numerous than in the normal thymus; many have erythrocytes or polymorphs in their degenerating centres, indicating that they have arisen round the remains of a blood-vessel (fig. 8, Pl. 6; cf. Jordan, **20**). The reticular cells of the corpuscles are indistinguishable from normal connective-tissue cells, such as can be seen invading the organ from the sheath, and also forming the adventitia of the blood-vessels (cf. fig. 24, Pl. 10, from another thymus).

18 Hours after Irradiation.

In this specimen, but not in any other, the two thymus lobes have partially fused; the whole organ is greatly reduced, and corresponds roughly in size to the medullary region of the normal thymus (fig. 20, Pl. 9). The large masses of nuclear debris filling the cortex in the last stage have almost completely disappeared. The edge of the section is occupied by branching connective-tissue cells, among which lie pycnotic and intact thymus nuclei (fig. 9, Pl. 6). The latter are easily distinguishable from those of the connective-tissue elements. Thymus cells are more common in the medulla, which occupies the greater part of the section, than at the edge, and pycnoses are rare among them. The destruction of thymus cells in the cortex and their persistence in the medulla has led to the apparent ' inversion ' of the two regions noted by other writers. The corpuscles of Hassall and cysts are similar to those seen in the preceding stage.

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[1] Drew ("Experimental Metaplasia", 'J. Exp. Zool.', 10) describes the formation of columnar ciliated epithelium from fibroblasts in Pecten, as the result of implanting pieces of ovary into the adductor muscle. The implant degenerates leaving a closed cyst lined by amitotically dividing fibroblasts; after 21–32 days these revert to an embryonic type, and afterwards become converted into columnar ciliated epithelium, which may persist for at least four months. The histological changes can be clearly followed, and they afford a good illustration of the potentialities of connective-tissue cells.

28 Hours after Irradiation.

As in the last thymus, the normal cortex has disappeared, but rather more small thymus cells have escaped injury. Fresh fibrous connective tissue, accompanied by capillaries, is penetrating the organ at all points from the surrounding sheath, and ingesting the few remaining pycnotic nuclei. Large blood-vessels and numerous corpuscles of Hassall can be seen, but cysts are rare, probably because resorption of the debris is nearly complete. Occasional mitoses are occurring which are too large to be those of thymus cells ; the latter are not dividing.

41–67 Hours after Irradiation.

During this period the thymus does not undergo any conspicuous change. Pycnotic nuclei are rare. Connective-tissue elements continue to invade the organ ; they form an irregular lighter-staining border and are pushing farther in among the persisting thymus cells ; some of these connective-tissue cells at the edge are dividing by mitosis.

3 Days 4 Hours after Irradiation.

This thymus shows no increase in size ; the peripheral region remains lighter-staining than the medulla, but the distinction between the two is not so marked as before, owing to the centripetal growth of the connective tissue. Corpuscles of Hassall can be found near the edge of the thymus apparently derived from the connective tissue which penetrated the organ after irradiation.[1] The first indications of regeneration can be found at this stage; the nuclei of the thymus cells scattered among the invading connective tissue at the edge have increased in size till the majority resemble the largest found in the normal thymus (fig. 10, Pl. 6; cf. figs. 1 and 9, Pl. 6). These nuclei do not stain as deeply with haematoxylin as those in the medulla, but can still be distinguished by their dark chromatin masses, from the adjacent pale nuclei belonging to the connective tissue. Mitoses corresponding in size to these nuclei are fairly common round the edge; similar mitoses, and also smaller ones, can be seen in the medulla, where no general enlargement of the thymus nuclei has taken place.

3 Days 19 Hours after Irradiation.

Thymus cells of the large type previously described have increased in number at the edge, and they continue to divide. Connective tissue still forms a considerable proportion of the organ, but the increase in the thymus cells tends to carry it inwards to the medulla. Corpuscles of Hassall are numerous, many of them occurring as before in the peripheral zone of the thymus; they are not confined to the medulla as in the normal organ.

4 Days 18 Hours after Irradiation.

In this thymus regeneration has proceeded far enough for a partial differentiation into a true cortex and medulla to have taken place (fig. 22, Pl. 9). This can be seen in both transverse and horizontal sections. It has been brought about by the centripetal movement of the connective tissue and vascular elements, and by the proliferation of persistent thymus cells, particularly those of the cortex. The nuclei of the thymus cells vary in size, but average larger than those in the normal organ. Mitoses are numerous in the cortex, but uncommon in the medulla. Differentiation of the cortex is farther advanced. Large cysts, some ciliated, are conspicuous at this stage; they appear to be derived from regressing vascular tissue; corpuscles of Hassall occur in the reconstituted medulla, but can no longer be found in the cortex.

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[1] Jaffe (17, fig. 1, Pl. 17) illustrates the formation of a corpuscle of Hassall in the peripheral region of a piece of the thymus of a guinea-pig (after autoplastic grafting) in an area extensively invaded by connective tissue.

5 Days 18 Hours after Irradiation.

This thymus, though only a day older than the last, is histologically reconstructed (fig. 23, Pl. 9); it has increased in size very considerably, and the cortical and medullary regions are well marked. No large blood-vessels can be seen at this stage. Mitoses are common in the cortex, where the thymus nuclei are still large. The connective-tissue and blood-vascular elements which invaded the organ immediately after irradiation are now regressing in the medullary regions. Ciliated cysts and corpuscles of Hassall are being formed from degenerating vascular tissue (figs. 25 and 26, Pl. 10); they are larger and more numerous than in the normal thymus medulla. Thin strands of fresh connective tissue can be seen pushing into the regenerated cortex (fig. 25, Pl. 10), but this invasion is far scantier than the last, and less conspicuous owing to the continued proliferation of thymus cells.

11½ Days after Irradiation.

This thymus resembles the normal organ more nearly than the last, since both corpuscles of Hassall and cysts are rare and there is little fibrous or vascular tissue invading the cortex (fig. 27, Pl. 10). The regression of the connective and blood-vascular tissue prominent in the X-rayed thymus has been rapid; the corpuscles of Hassall and cysts conspicuous in the newly regenerating thymus (figs. 23 and 25, Pls. 9 and 10) represented late stages of the process. The degenerated tissue has been replaced in the medulla by the fresh connective and blood-vascular elements which were pushing into the cortex in the last stage, these having been carried centripetally inwards like their predecessors. Mitoses are very numerous; the thymus nuclei vary in size, but approximate to those in the normal organ. Scattered pycnoses are not uncommon.

DISCUSSION.

The above account of degenerative changes in the irradiated thymus resembles that of Regaud and Crémieu (**22**), but although these writers describe the hypertrophy both of corpuscles of Hassall and also of connective-tissue and blood-vascular elements, they continue to regard the reticular cells forming the corpuscles as epithelial, though offering no positive evidence in support of this theory.[1] Jolly, in his 'Traité technique d'Hématologie' (published 1923), adopts a similar view; he classes the thymus as a lymph-organ and explicitly rejects the possibility of a vascular or connective-tissue origin for the corpuscles of Hassall; he describes and illustrates cysts arising through the confluence of several corpuscles, and regards these formations also as products of reticular epithelial cells, and centres of their involution.

Winiwarter, in a critical article on the histology of the thymus (**24**), concludes for various reasons that the corpuscles of Hassall are of epithelial origin, though he agrees with Dustin that the thymus cells are not lymphocytes.

Jordan and Horsley (**20**), however, in a recently published note on the thymus of man and the rabbit, state that 'concentric corpuscles arise chiefly from hypertrophied endothelial cells of precapillary arterioles and the immediately investing reticulum cells'. These authors have found structures closely comparable to Hassall's corpuscles in atrophic subcutaneous lymph-nodes in the rabbit.

As described above the present material offers conclusive evidence of the development of both corpuscles of Hassall and cysts from the blood-vessels and their investing connective tissue (fig. 8, Pl. 6; figs. 18, 19, Pl. 8; fig. 21, Pl. 9; figs. 24, 26, Pl. 10). It is probable that the periodic degeneration of blood-vessels in the normal thymus to form corpuscles of Hassall is the cause of the numbers of free erythrocytes found not infrequently among the thymus cells.

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[1] The present writer has not seen a copy of the more detailed account of this work published by Crémieu (**4**); portions of it have been quoted and criticized by Dustin (**6**).

Irradiation has the effect of increasing considerably the numbers of cysts and concentric corpuscles, but the process by which these develop appears to be perfectly normal. The cavity of a cyst may be that of a blood-vessel which has broken down, or a space filled with degenerating thymus cells ; in either case it becomes lined by perithelial connective tissue, the cells of which are not damaged by the X-rays. Probably the majority of the smaller blood-vessels in the thymus undergo change, but their loss is made good by the fresh blood-vascular tissue which simultaneously invades the organ from the sheath. The cysts are transitory structures, and have mostly disappeared by the time resorption of the thymus cell debris is complete. A new series of cysts and large corpuscles of Hassall arises later (4–6 days after irradiation, fig. 23, Pl. 9; fig. 25, Pl. 10), when the cortex of thymus cells has been reconstituted, coincidently with the regression of the additional vascular tissue which penetrated the organ during its involution. This new series becomes resorbed in its turn, and six days later corpuscles of Hassall and cysts are no more numerous than in the normal thymus.

The re-establishment of the medulla can be clearly traced in this material (figs. 22, 23, Pl. 9; fig. 25, Pl. 10); during the intense proliferation of thymus cells in the cortex, the connective-tissue and blood-vascular elements which invaded it after irradiation move centripetally, becoming aggregated in small areas near the middle of the organ. No fresh proliferation of epithelial elements can be distinguished during medulla formation, as would be expected from the lympho-epithelial theory of the thymus. The reconstitution of the medulla in the regenerating organ by the ingrowth of mesodermal cells suggests strongly that these areas are so formed in the embryonic thymus, and that they should not be regarded as condensations of the original epithelium.

Proliferation of the connective tissue and walls of the blood-vessels accompanied by degeneration and scarcity of the thymus cells, and the contrasting condition, have been previously described and discussed by Dustin, as seasonal variations in the frog, and also under experimental conditions in both the frog and the mouse (**6**, **8**, and **9**).[1]

Although the derivation of both corpuscles and cysts from the blood-vascular tissue can be clearly traced, it is impossible to write with complete certainty of the exact part played in these transitions by endothelium, connective tissue, and smooth muscle. Dustin (**5**) has described cysts in the thymus of the axolotl which are similar in almost every detail to those in the thymus of the mouse; their formation and the nature of the cells from which they arise is discussed at some length, and the conclusion is reached that these cells, which may divide amitotically, all belong to the connective-tissue group. Dustin regards the cells adjacent to the blood-vessels in the frog's thymus as of the same type; these, too, undergo irregular cleavage after autoplastic grafting (**6**).

Carleton and Florey (**3**) in a recent paper discuss the difficulty of distinguishing smooth muscle from connective tissue in the mammalian lacteal; they state that nuclear lines and cleavages (present in cells which have ceased to divide mitotically and suggestive at first sight of amitosis)[2] are characteristic of smooth-muscle nuclei and serve when present to distinguish them from those of connective tissue.

In the thymus both kinds of tissue would normally be present in the walls of the blood-vessels, and might be expected to proliferate under the influence of the same stimulus; it is noticeable that mitoses are absent among the cells which form the cysts at a time when they are increasing rapidly in number. Lines of

[1] Dustin (8 and 9): these papers on the thymus of the mouse are short and do not include illustrations.

[2] It is recognized that the question of amitosis is controversial, and that it is almost impossible to obtain conclusive evidence of its occurrence. In the mouse thymus after irradiation the increase in the connective-tissue elements, within a few hours, is so conspicuous, that a consideration of the appearance of the nuclei and the absence of mitotic figures (especially among the perithelial cells lining the newly formed cysts), leads almost inevitably to the inference that cell division is taking place amitotically.

cleavage and other indications of amitotic divisions can be clearly seen in the nuclei of these cells (figs. 6 and 7, Pl. 6; cf. fig. 3, Pl. 6), which appear to be all of one type though some develop cilia and others do not. This capacity to develop cilia renders it unlikely that they are specialized contractile cells, though some of the nuclei in process of cleavage resemble the smooth-muscle nuclei depicted by Carleton.

On the whole the evidence from the mouse supports Dustin's conclusions that the cells forming the cysts belong to the connective-tissue group, although it is possible that there are smooth-muscle cells among them which have reacted similarly to the irradiation. The endothelial cells appear to degenerate when the cyst is formed actually in the cavity of a blood-vessel (figs. 18, 19, Pl. 8; fig. 26, Pl. 10), and their nuclei can sometimes be found in the lumen.

Jaffe (**17**) mentions rapid amitotic division of 'reticulum epithelial' cells four days after autoplastic grafting in the thymus of the guinea-pig. In another paper (**16**) he notes proliferation of the 'reticular' cells, which are at first spindle-shaped but become epithelioid—an indication of their connective-tissue nature.

The absence of a continuous epithelial reticulum, such as is postulated by advocates of the lympho-epithelial theory, can be clearly demonstrated in sections through a thymus fixed ten hours after irradiation, when the mass of cortical thymus cells are degenerating; there are so few large cells in the cortex similar to those in the medulla (which are untouched by the X-rays), that they cannot possibly be said to constitute a reticulum (fig. 16, Pl. 8). The cells forming a border round the reduced thymus, which have been described by other observers as epithelial or epithelioid and have been erroneously regarded as a condensation of pre-existing reticular cells, are fibroblasts. They can be seen shortly after irradiation pushing in from the surrounding sheath and resorbing the pycnotic thymus nuclei (fig. 4, Pl. 6; fig. 13, Pl. 7; fig. 21, Pl. 9). Jaffe and others (**14, 16, 17, 21**) appear to have confused these connective-tissue cells and the enlarged 'epithelioid' thymus cells which by repeated division reconstitute the cortex in the regenerated thymus; just before their period of mitotic activity the nuclei of these cells become enlarged till they are comparable to those of the connective-tissue cells among which they lie (fig. 10, Pl. 6). Similar nuclei having a diameter larger than the average can be found among the small thymus cells of the normal organ. If these latter are the products of the original epithelium of the pharynx, their regeneration from larger 'epithelioid' cells may be considered a repetition of the embryonic process.

This experimental study of the histology of the thymus fails completely to support the lympho-epithelial theory of its structure, commonly expounded in the text-books. No epithelial elements can be distinguished in the thymus, other than the larger thymus cells. Both corpuscles of Hassall and ciliated cysts are derived from the blood-vessels and accompanying connective-tissue. It will be seen below that these conclusions are reinforced by a study of sections through tissue cultures of cat thymus.

Observations on Tissue Cultures of the Thymus of the Cat.

The changes occurring in pieces of thymus taken from advanced cat embryos and cultivated in plasma, were studied in order to throw light on the nature of the thymus reticulum and the corpuscles of Hassall.

Pieces of thymus, 1 mm. or less in diameter and about 0·5 mm. thick, were incubated for 1–13 days in maternal plasma, without subculturing or the addition of embryo extract. The method employed was that of tube cultures previously described by Carleton (2), but a few cover-slip preparations were also made for comparison with the others. At intervals of 24 hours or more the implants were fixed in Bouin's fluid, diluted with two parts of distilled water to one of the fixative; they were subsequently embedded in paraffin and sectioned at 6μ. Mitoses were occurring at the time of fixation in all cultures included in this description (fig. 31, Pl. 11). Control pieces of thymus were similarly fixed without incubation.

An examination of sections through the latter showed that the thymus was of the adult type and contained conspicuous corpuscles of Hassall, all lying in the medullary region of the lobules; the latter were small and separated by connective-tissue septa containing capillaries. In the medullary regions reticular cells were sparsely distributed, and the blood-vessels small and inconspicuous (fig. 28, Pl. 11).

After 24 hours incubation conspicuous changes have occurred in the tissue; many of the small thymus cells are degenerating, including the few which have emigrated into the surrounding plasma. Connective tissue has proliferated until it forms an almost complete fibrous sheath round the implant; mitoses are occurring among the cells with large nuclei, but the spindle-shaped cells in the walls of the blood-vessels appear to be cleaving amitotically, as in the X-rayed thymus (fig. 11, Pl. 6). Groups of reticular cells, indistinguishable from large connective-tissue fibroblasts, can be seen in the medulla surrounding corpuscles of Hassall; such cells are aggregated together unlike those in the normal medulla, with relatively few thymus cells lying together and those pycnotic (fig. 29, Pl. 11). The reticular cells of the medulla and the connective-tissue cells derived from the interlobular septa are both engaged in phagocytosis of the degenerating thymus cells. Corpuscles of Hassall only occur in the medullary region.

Sections of older cultures show a continuation of the process initiated 24 hours after implantation, multiplication of fibrous connective tissue and pycnosis of the small thymus cells. The connective tissue forms a complete sheath 'round the implant, and a few cells branch out into the surrounding plasma (fig. 32, Pl. 12). Three days after implantation corpuscles of Hassall can be seen at the edge of the tissue on either side a degenerating central region containing pycnotic thymus cells. Their position clearly indicates that they have been formed since the tissue was incubated; in the normal thymus the lobules are small and the corpuscles of Hassall always situated in the medulla. Cultivation in plasma has brought about a reversal of the normal dark- and light-staining cortical and medullary regions, owing to the proliferation of connective tissue round the edge. The corpuscles of Hassall embedded in this connective tissue, and continuous with it, are similar in all respects to normal ones (fig. 33, Pl. 12). In a 5-day culture corpuscles of Hassall can be found in isolated bays projecting from the implant into the plasma. In the two oldest cultures (13 days) corpuscles of Hassall persist in connective tissue, but the small thymus cells have all degenerated.

The above account does not agree with that recently published by Popoff (**21**), who gives neither illustrations nor complete details of his technique. He distinguishes certain reticular cells as epithelial, since, unlike connective-tissue cells, they do not store carmine and vital dyes. My own tissue cultures were made before Popoff's paper was published, and I have not repeated the latter's experiment of adding carmine to the plasma. The sections through the cultures of the cat's thymus show plainly that it is connective tissue that forms a border round the implant and proliferates in the medulla, and I am unable to accept Popoff's statement that these cells are epithelial.[1] This 'epithelium' he describes as forming strands and islets, sometimes showing intercellular and intracellular vacuoles; such vacuoles are common in the connective tissue of my cultures.

Corpuscles of Hassall are normally small in the rabbit thymus, which was the material used by Popoff; their formation is more readily observable in the thymus of the cat.

The conclusions which can be drawn from a study of cultures of the cat's thymus may be summarized as follows: the reticular cells of the medulla resemble the large fibroblasts of the interlobular connective tissue and behave similarly; the cells forming a border round the implant are also connective-tissue cells, and it is impossible to distinguish any epithelial reticulum. New corpuscles of Hassall arise at the edge of the cultures, and their outer cells are continuous with those of the connective tissue which surrounds them; these corpuscles of Hassall may persist after all the thymus cells have degenerated.

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[1] It is possible that the non-phagocytic cells which Popoff believes to belong to an epithelial reticulum are large thymus cells, such as occur normally. The addition of the bone marrow or embryo extract to the culture may have caused enlargement of the peripheral thymus cells of the implant and a consequent increase in the epithelioid type; cf. the enlargement of thymus cells in the thymus regenerating after irradiation.

I wish to express my thanks to Dr. H. M. Carleton, without whose assistance I should have been unable to make these tissue cultures. The work was done in the Department of Physiology at Oxford.

OBSERVATIONS ON THE EFFECT OF X-RAYS ON THE LYMPH NODES OF THE MOUSE.

The lymph nodes are much less affected by the action of X-rays than is the thymus. Six hours after irradiation those adjacent to the thymus contain a number of pycnotic nuclei, scattered fairly evenly throughout their tissue; these are being phagocyted by neighbouring cells. Seventeen hours after irradiation pycnotic nuclei are rare; owing to the destruction of a number of lymphocytes the tissue of the node appears less dense than usual, but there are no conspicuous changes. The reticular tissue of the capsule shows no sign of proliferation; mitoses are rare. Twenty-four hours later mitoses of the large type are fairly common, and the lymph node has regained its normal appearance.

Although occasional groups of undegenerating reticular cells can be found both in the normal and in the irradiated lymph nodes of the mouse, corpuscles similar to those in the thymus do not arise, as they are said to do, in the rabbit (**20**). It is possible that their absence is connected with the meagre vascularization of the lymph node, as well as with its different structure and mode of development.

SUMMARY.

1. A number of male mice 4–6 weeks old were subjected to irradiation and killed at intervals of 2 hours to 12 days after the treatment. A detailed study was made of the resulting histological changes in the thymus. Immediately after irradiation most of the small thymus cells comprising the cortex of the

organ degenerate. Simultaneously the existing connective-tissue elements in the thymus proliferate amitotically, and fresh connective tissue and blood-vessels invade the periphery of the organ from its sheath. Numerous ciliated cysts develop in the thymus, derived from the blood-vessels and their adjacent connective tissue; the majority of these disappear 28 hours after irradiation when resorption of the cell debris is complete.

Regeneration is initiated 3 days after irradiation by an enlargement of the nuclei of the thymus cells persisting at the edge of the organ; these, by repeated division, reconstitute the cortex. Fresh medullary areas are formed by the aggregation of the connective tissue and blood-vessels which are carried centripetally during regeneration; most of this tissue degenerates, giving rise to a fresh series of corpuscles of Hassall and cysts. These are transitory structures and have almost all disappeared from the medulla by the time histological regeneration of the thymus is complete, 12 days after irradiation.

2. Small pieces of thymus from an advanced cat embryo were cultivated in normal maternal plasma; the implants were fixed after various periods of incubation, embedded in paraffin and sectioned. The histological changes after incubation are similar to those in the irradiated thymus, before the latter begins to regenerate; they include pycnosis of the small thymus cells and proliferation of fibrous connective tissue. This forms a border round the implant in which fresh corpuscles of Hassall develop, their outer cells being continuous with the fibrous connective tissue.

3. The significance of these results with regard to the normal histology of the thymus is discussed, as are the conclusions of other workers who have studied similar changes in the thymus after experimental involution. Both these experimental studies of the histology of the thymus indicate the complete absence of an epithelial reticulum, such as is frequently postulated; the corpuscles of Hassall, and cysts generally associated with it, are derived from the blood vascular and accompanying connective tissue.

4. A note is added on the reaction of typical lymph nodes to

the irradiation; this is slight in comparison with that of the thymus.

I wish to express my thanks to Professor J. P. Hill, F.R.S., for his advice, and to Dr. A. S. Parkes for supplying me with the irradiated mice. With the exception of the tissue cultures, the work was carried out in the Department of Anatomy and Embryology, University College, London.

Dec. 1927.

LITERATURE CITED.

1. Aubertin et Bordet.—" Action des rayons X sur le thymus ", ' C. R. Soc. Biol.', 1909, 1, p. 1091.
2. Carleton (1925).—" Growth, Phagocytosis, and other Phenomena in Tissue Cultures of Foetal and Adult Lung ", ' Phil. Trans. Roy. Soc.', B, 213.
3. Carleton and Florey (1927).—" The Mammalian Lacteal ", ' Proc. Roy. Soc.', B, 102.
4. Crémieu (1912).—' Étude des effets produits sur le thymus par les rayons X ', Lyon, 1912.
5. Dustin (1911).—" Le Thymus de l'Axolotl ", ' Arch. de Biol.', 26.
6. —— (1913).—" Recherches d'histologie normale et expérimentale sur le thymus des Amphibiens Anoures ", 1re partie, ' Arch. de Biol.', 28.
7. —— (1920).—Ditto, 2ième partie, ibid., 30.
8. —— (1923).—" Formation de cavités a épithélium cilié aux dépens de tractus vasculaires dans le thymus de la souris ", ' C. R. de l'Ass. des Anat.', 20.
9. —— (1925).—" Influence des variations brusques du pH sur le thymus et les organes lymphopoétiques ", ibid.
10. —— (1927).—" Étude comparative entre l'action des radiations et l'action des poisons caryoclasiques ", ' Strasbourg Médical ', 85ième année, 1.
11. Firket et Linhoff (1925).—" Sur l'action des injections intrapéritonéales d'huile de paraffine et de solutions huileuses d'oléate ferreux sur les tissues lymphoïdes, le thymus et les séreuses peritonéales ", ' C. R. Soc. de Biol.', 1925, 1.
12. Goldner (1923).—" Action d'adrénaline sur le thymus ", ibid., 1923.
13. —— (1925).—" Reaktionen der Thymus während der Knochenbruche ", ' Arch. mik. Anat. u. Entw. mech.', 104.
14. Gottesmann and Jaffe (1926).—" Studies on the Histogenesis of Autoplastic Thymus Transplantations ", ' Journ. Exp. Med.', 43.
15. Hammar (1921).—" The new views as to the Morphology of the Thymus Gland, and their bearing on the Problem of the Function of the Thymus ", ' Endocrinology ', 5.

16. Jaffe and Plavska (1925).—" Experimental studies on the formation of Hassall's corpuscles ", ' Proc. Soc. Exper. Biol. and Med.', **23.**

17. Jaffe (1926).—" Studies on the Histogenesis of Autoplastic Thymus Transplantations ", ' Journ. Exp. Med.', **44.**

18. Jolly (1925).—" Involution aiguë du thymus produite par une injection d'alcool.", ' C. R. Soc. de Biol.', 1925.

19. —— (1923).—' Traité technique d'Hématologie ', **2.**

20. Jordan and Horsley (1927).—" The significance of Hassall's corpuscles ", ' Anat. Rec.', **35.**

21. Popoff (1926).—" The Histogenesis of the Thymus as shown by Tissue Cultures, Transplantation, and Regeneration ", ' Proc. Soc. Exper. Biol. and Med.', **24.**

22. Regaud et Crémieu (1911).—" Évolution des corpuscles de Hassall dans le thymus röntgenisé du chat, &c.", ' C. R. Soc. de Biol.', 1911, **2** ; 1912, **1.**

23. Rudberg (1907).—" Stüdien über die Thymusinvolution. Die Involution nach Röntgenbestrahlung ", ' Arch. für Anat. und Phys.', 1907 (Anat. Suppl.).

24. Winiwarter (1924).—" Le Thymus est-il un organe lymphoïde ? " ' Bull. d'Hist.', **1**, p. 11.

ADDENDUM.

Since this paper was completed, a further paper has been published by Popoff :

> " The Histogenesis of the Thymus as shown by Tissue Cultures ", ' Arch. f. exp. Zellforsch.", **4**, 1927.

This is a detailed account of the work referred to above (**21**). Lack of space prevents a full criticism, but the following points may be noted, in addition to previous comments.

The author assumes that the lympho-epithelial theory of the nature of the thymus has been proved by the embryological studies of Maximow and Hammar ; actually these have been severely criticized and not confirmed by later investigators such as Dustin. The latter has accumulated a formidable body of evidence against the lympho-epithelial theory, which cannot lightly be disregarded. Popoff, however, assumes the existence of an epithelial thymus reticulum, and endeavours to confirm Maximow's theory by a study of tissue cultures ; previous investigators in this field have failed to reach agreement.

Maximow's cover-slip technique was employed, and cultures of rabbit thymus were examined in vitro, and subsequently fixed, sectioned, and stained with haematoxylin and eosin-azur II. Popoff presents a detailed analysis of the constituent cells of the cultures, including those related to the blood-vessels, which are clearly mesodermal. Curiously enough, he makes no mention among these of the large connective-tissue cells, with pale oval vesicular nuclei, which are prominent in my own cultures; reference to Popoff's description and figures, particularly figs. 1 and 4, indicates that these cells are described as epithelial, on the ground that they do not actively ingest carmine. Cells morphologically similar to these, with the characteristic staining reactions of connective tissue, occur in the normal thymus, both in the interlobular clefts and medullary regions. After 24 hours cultivation in plasma these cells are far more prominent (figs. 29 and 30), considerable proliferation, not condensation, having taken place. No difference can be distinguished between those forming isolated medullary islands, often round corpuscles of Hassall, and others belonging to the interlobular clefts, which are unmistakably connective-tissue cells.

Figs. 32 and 33 in this paper afford evidence that the prominent cells with round vesicular nuclei, which other writers have described as epithelioid, are large connective-tissue fibroblasts, which participate in the formation of the characteristic corpuscles of Hassall. It appears to the present writer that a study of tissue cultures of mammalian thymus not only lends no support to the lympho-epithelial theory of the thymus, but renders it no longer tenable.

EXPLANATION OF PLATES 6–12.

All illustrations are from haematoxylin-stained sections, unless it is otherwise stated. The drawings on Pl. 6 were all made to the same scale with the aid of a camera lucida. I am indebted to Mr. F. Pittock of the Department of Anatomy, University College, for the remaining illustrations, which are untouched microphotographs. Figs. 1–10 and 12–26 inclusive are taken from transverse sections through the thymus of male mice of approximately the same age.[1]

ABBREVIATIONS.

b.v., blood-vessel; *b.v.l.*, lumen of blood-vessel; *c.*, cilia; *cy.*, cyst; *c.cy.*, ciliated cyst; *cap.*, capillary; *coll.d.*, collagenous debris; *c.per.*, ciliated perithelial cell; *c.t.*, connective tissue; *c.t.s.*, connective-tissue sheath; *cor.*, cortex; *c.H.*, corpuscle of Hassall; *d.c.*, degenerating centre; *d.pm.*, degenerating polymorph; *end.*, endothelial cell; *e.t.c.*, enlarged thymus cell; *f.*, fibrous connective tissue; *i.c.t.*, interlobular connective tissue; *m.*, medulla; *mit.*, mitosis; *mn.*, mononuclear leucocyte; *n.c.*, nucleus with cleavage lines; *n.e.*, epithelioid nucleus; *n.p.*, pycnotic nucleus; *n.d.*, degenerating nucleus; *pm.*, polymorph; *per.*, perithelial cell; *r.c.*, reticular cell; *t.c.*, thymus cell.

PLATE 6.

Fig. 1.—Normal 7 weeks mouse; group of cortical thymus cells showing the variation in the size of their nuclei.

Fig. 2.—Normal 7 weeks mouse; two corpuscles of Hassall with their adjacent cells.

Fig. 3.—Normal 7 weeks mouse; ciliated cyst with thymus cells in the lumen; note lines of cleavage in the nuclei of the cells bordering the cyst.

Figs. 4 and 5.—6 hours after irradiation; enlargements of parts of fig. 13, which shows pycnosis of thymus cells and invading polymorphs and epithelioid connective-tissue cells. Figs. 5 and 6 illustrate types of connective-tissue nuclei, some with irregular cleavages and others rounded and epithelioid.

Fig. 6.—10 hours after irradiation; perithelial cells of a capillary in process of forming a ciliated cyst; at x degenerated cells are being extruded into the lumen.

Fig. 7.—10 hours after irradiation; part of the wall of a cyst; the condition of the nuclei bordering the cyst indicates that they are proliferating amitotically; a strand of cells similar to those at x connects the cyst at that point with the outer cells of a large corpuscle of Hassall (not drawn). The nuclei of adjacent thymus cells are shrunken and pycnotic.

Fig. 8.—10 hours after irradiation; formation of a corpuscle of Hassall from a blood-vessel.

Fig. 9.—28 hours after irradiation; group of cells from the edge of the reduced thymus, consisting of intact small thymus cells and larger connective-tissue cells which have invaded the organ after irradiation.

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[1] In the course of reproduction the plates were unfortunately reduced unequally; illustrations previously on the same scale are now shown with somewhat different magnifications, e. g. figs. 15 and 17.

Fig. 10.—3 days 4 hours after irradiation; similar to the above, showing enlargement of persistent thymus cells in the cortex and mitoses occurring among them. At this stage the thymus cells may be described as epithelioid.

Fig. 11.—From a section through a piece of cat thymus after incubation for one day; irregular cleavage in cells adjacent to a blood-vessel (cf. figs. 4 and 5).

PLATE 7.

Fig. 12.—Normal 7 weeks mouse; general view of thymus to show cortical and medullary areas. × 56.

Fig. 13.—6 hours after irradiation; pycnosis of thymus cells and invasion of polymorphs and epithelioid connective-tissue cells. Figs. 4 and 5 show parts of this photograph in greater detail at the levels indicated. × 430.

Fig. 14.—10 hours after irradiation; the degeneration of masses of thymus cells has already brought about a reduction in size; cortical and medullary thymus areas are becoming indistinct in the middle of the organ; numerous large blood-vessels can be seen. × 56.

Fig. 15.—Enlarged view of cyst shown in fig. 14; note ciliated border and cell debris in the lumen. × 715.

PLATE 8.

Fig. 16.—10 hours after irradiation; mass degeneration of thymus cells and absence of reticulum in the cortex; in the medulla, enlargement of blood-vessels and persistence intact of majority of small thymus cells. × 215.

Fig. 17.—10 hours after irradiation; cyst being formed by the degeneration of the central part of a corpuscle of Hassall. × 650.

Fig. 18.—18 hours after irradiation; cyst formed by degenerated blood-vessel, containing collagenous debris and some nuclei; well-marked cilia have been developed by some of the perithelial cells of the border. Adjacent to the large cyst are smaller ones formed by the degeneration of thymus cells. × 650.

Fig. 19.—Similar to the above; cilia have been developed along the track of a degenerated blood-vessel. × 650.

PLATE 9.

Fig. 20.—18 hours after irradiation; low-power view of transverse section for comparison with fig. 12; note reduction in size, 'inversion' of cortex and medullary regions, and increase in blood-vascular tissue. × 55.

Fig. 21.—Part of the edge of fig. 20, indicated at x, under higher magnification, showing fibrous proliferation, cysts, and a corpuscle of Hassall still in continuity with the blood-vessel from which it has developed. × 412.

Fig. 22.—4 days 18 hours after irradiation; low-power view of a transverse section through the thymus to show partial redifferentiation of a medullary region. × 55.

Fig. 23.—5 days 18 hours after irradiation; similar to the above, showing that regeneration of both cortical and medullary areas has been accomplished. Note cysts and corpuscles of Hassall appearing as lighter patches in the medulla. ×55.

PLATE 10.

Fig. 24.—5 days 18 hours after irradiation; corpuscle of Hassall, forming from a blood-vessel, with degenerating polymorphs at its centre (cf. fig. 8). × 696.

Fig. 25.—5 days 18 hours after irradiation; as fig. 23; note aggregation of connective-tissue and blood-vascular elements to form medullary areas; corpuscles of Hassall are numerous and darkly stained; a large ciliated cyst has been formed by a degenerating blood-vessel. Mallory's connective-tissue stain. × 55.

Fig. 26.—Large cyst from fig. 25. × 696.

Fig. 27.—11½ days after irradiation; part of a horizontal section through the thymus to show decrease in blood-vascular and connective-tissue elements, both at the periphery and in the medullary regions. Staining as fig. 25. × 55.

PLATES 11 AND 12.

Figs. 11, 28, 29, 30, 32, and 33 represent pieces of thymus from a single cat embryo, fixed immediately (fig. 28), and after different periods of incubation. Fig. 31 is taken from a section through a similar piece of thymus after incubation.

Fig. 28.—Section through a piece of thymus from an advanced cat embryo, fixed without incubation; it shows the small lobules, each consisting of cortex and medulla, and the connective-tissue septa between them. × 50.

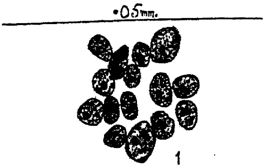
Fig. 29.—After incubation for one day; proliferation of fibrous connective tissue round a corpuscle of Hassall and pycnosis of thymus cells. × 375.

Fig. 30.—After incubation for one day; overgrowth of interlobular connective tissue. × 210.

Fig. 31.—After 4 days incubation; a corpuscle of Hassall has developed in the peripheral zone of the culture; some of the connective-tissue cells are dividing by mitosis. × 620.

Fig. 32.—After five days incubation; general view of implant showing border of fibrous connective tissue, containing corpuscles of Hassall, and degeneration of the majority of small thymus cells. × 188.

Fig. 33.—Large corpuscle of Hassall from fig. 32, showing continuity of its outer cells with the surrounding fibrous tissue. × 646.



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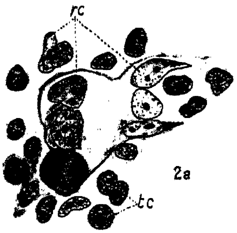

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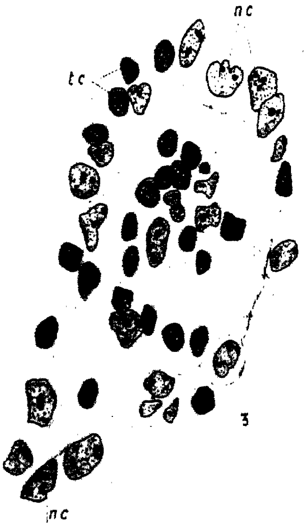

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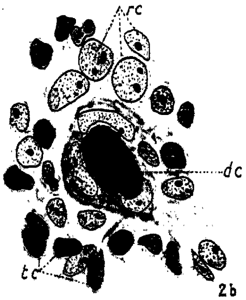

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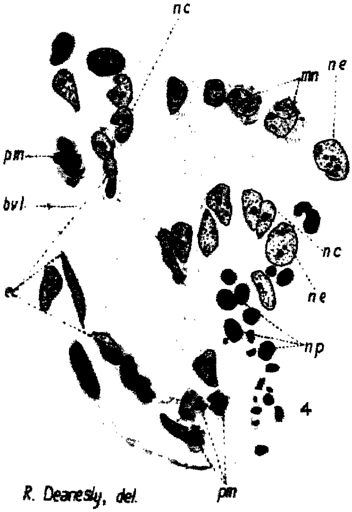

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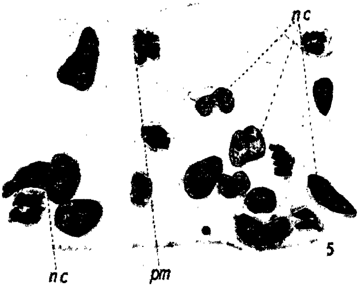

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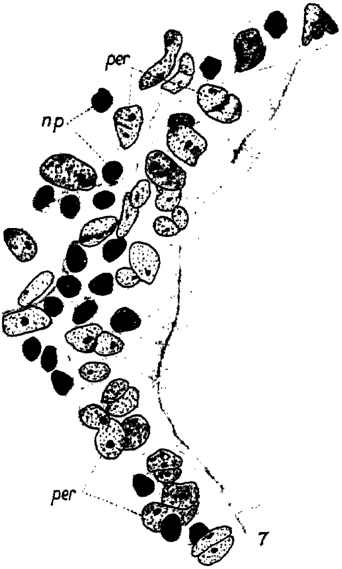
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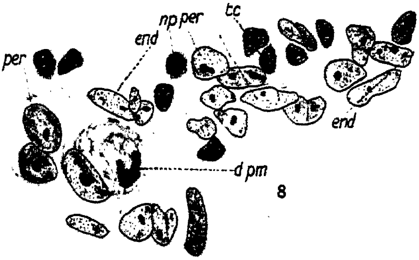
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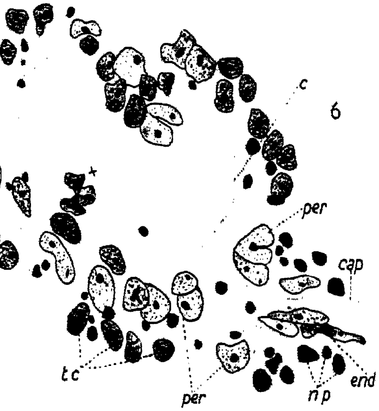
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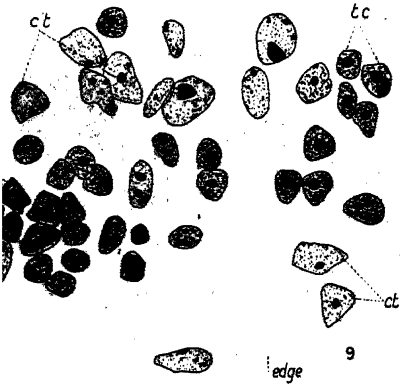
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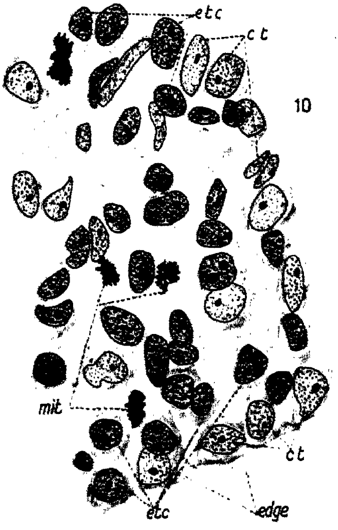
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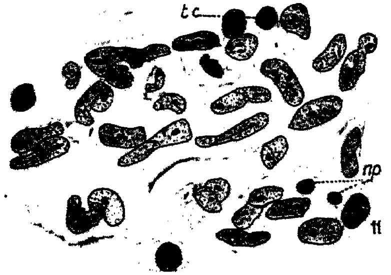
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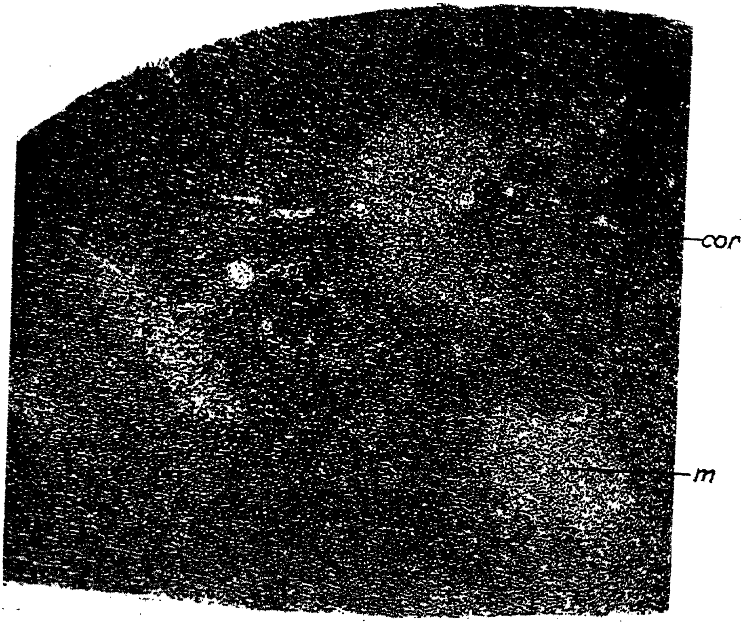

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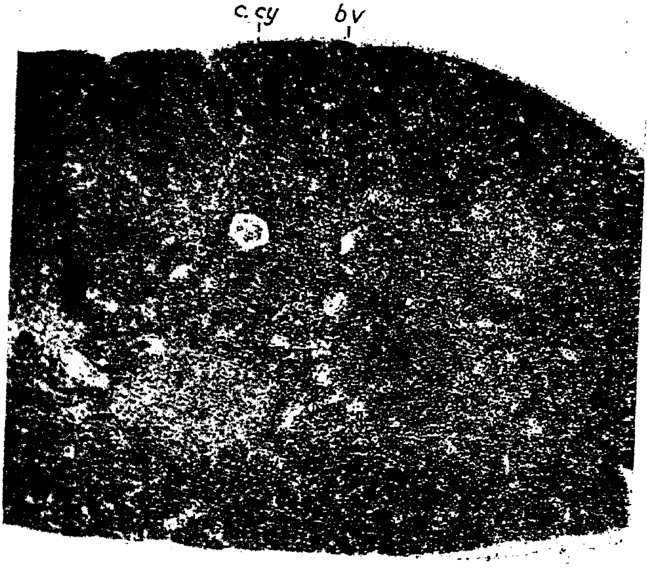

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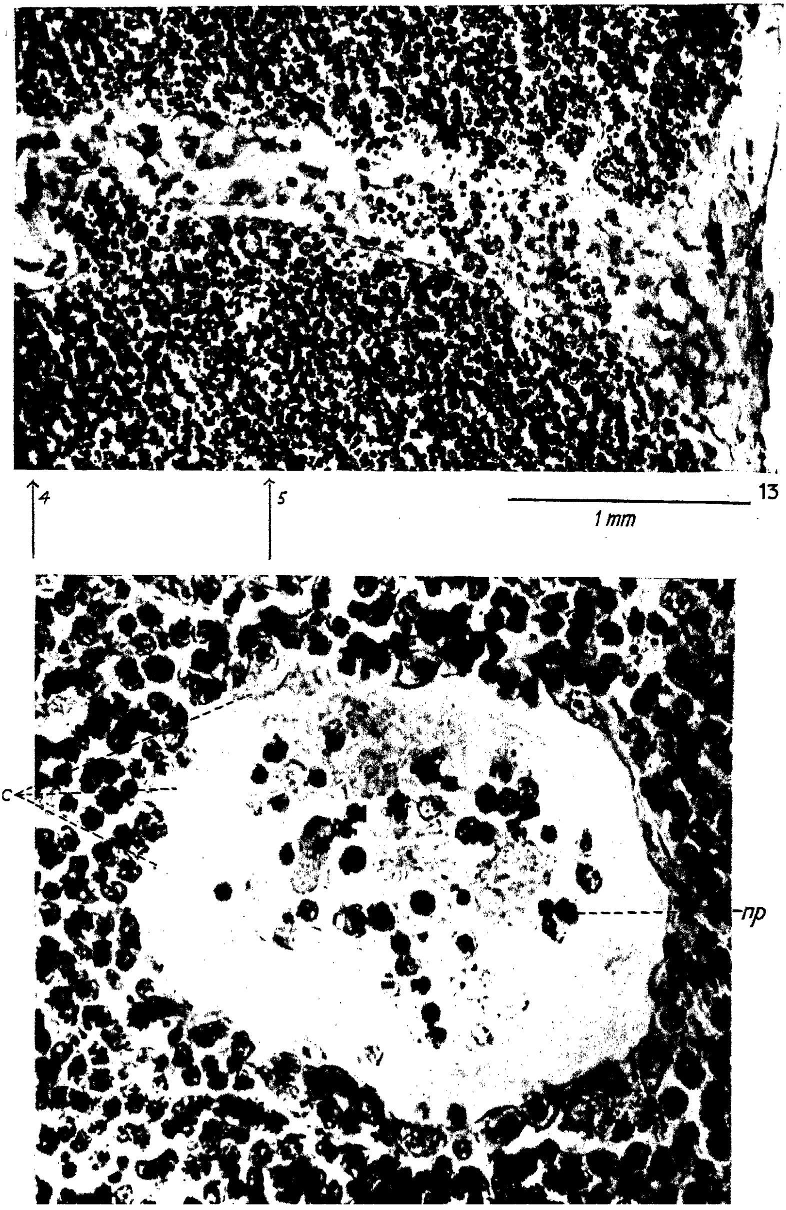

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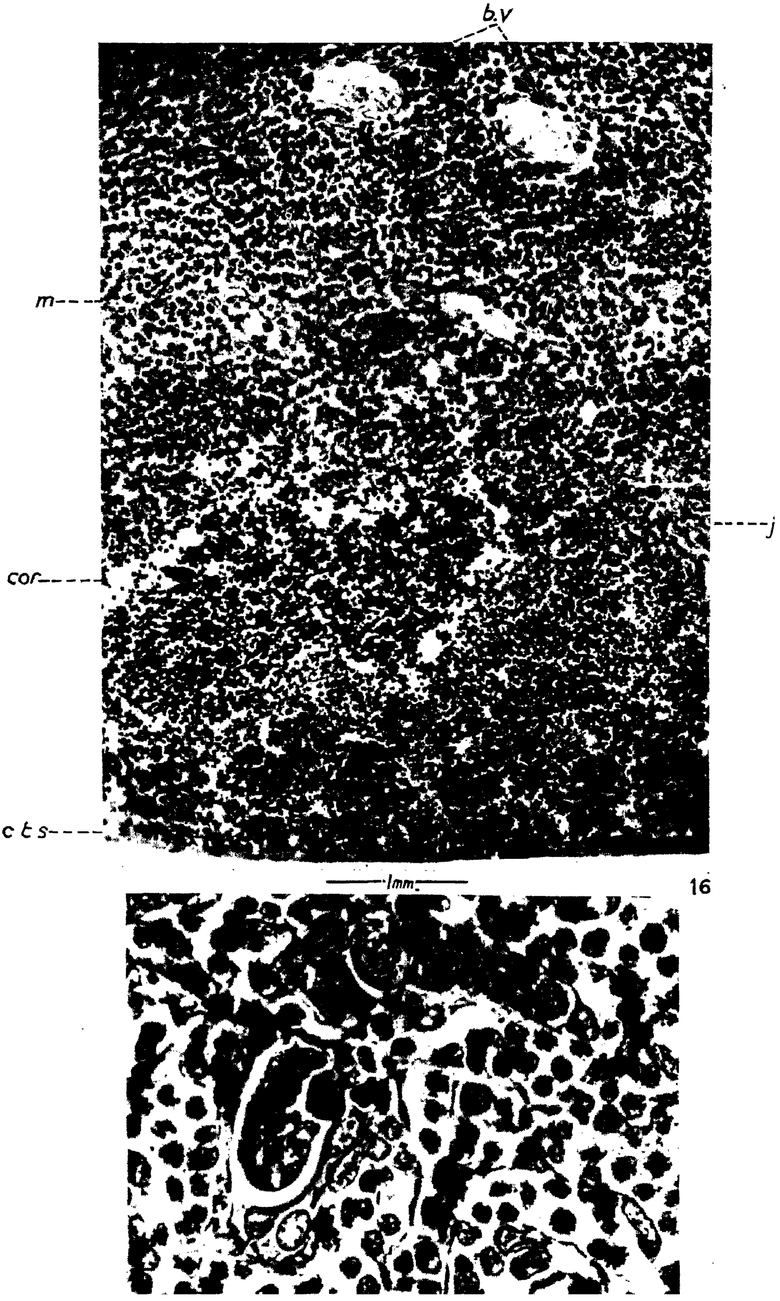

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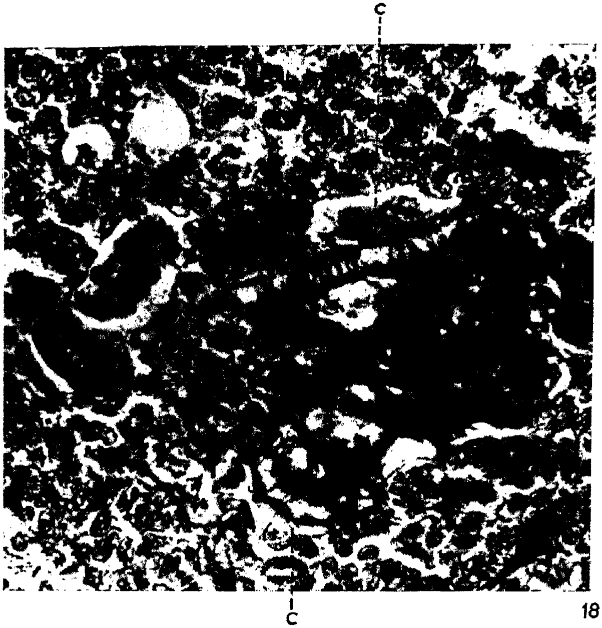

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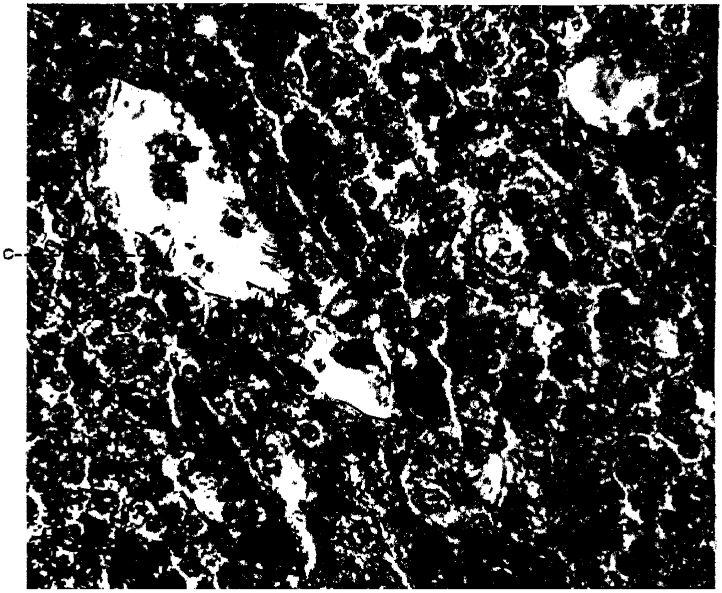

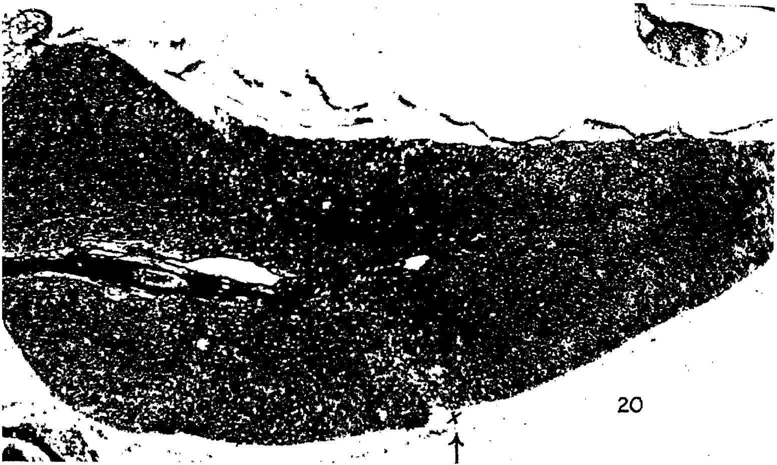

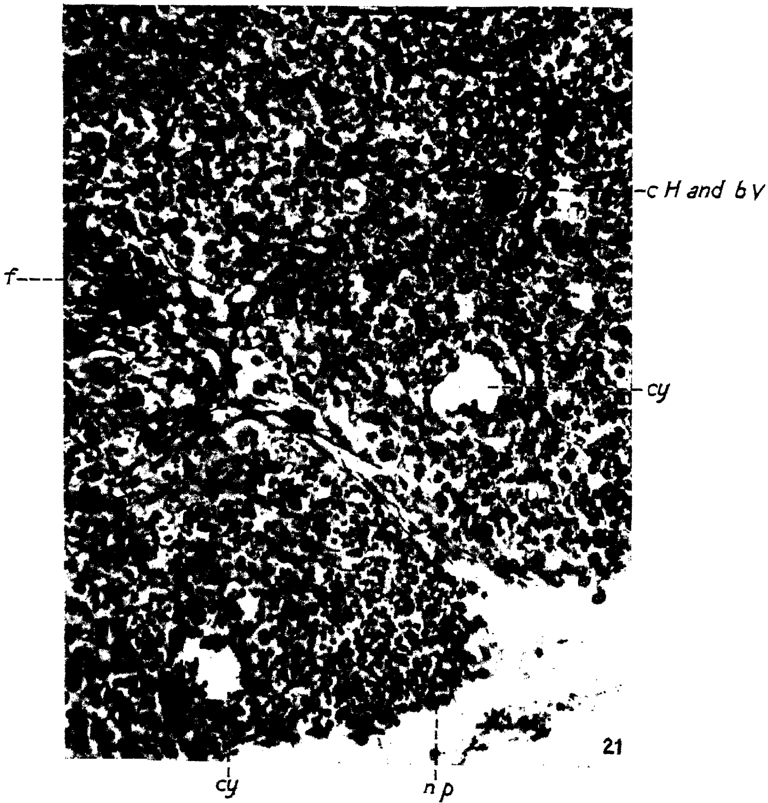

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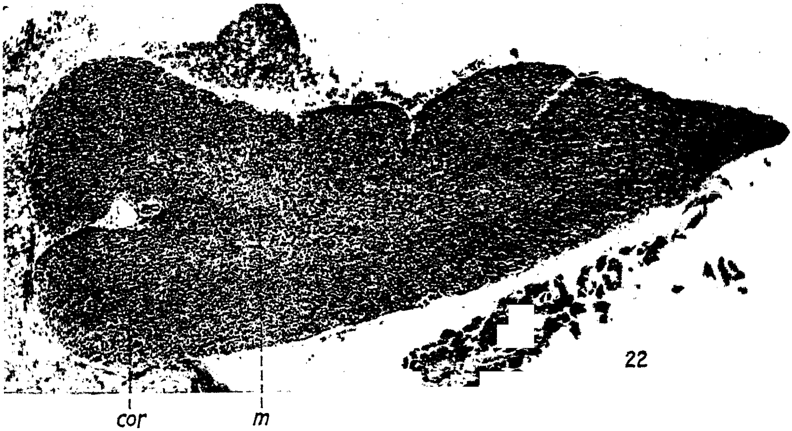
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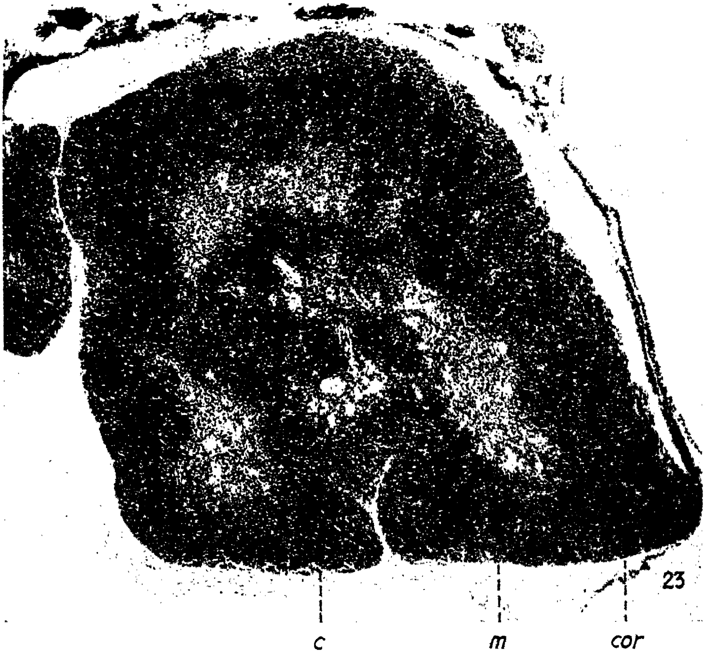
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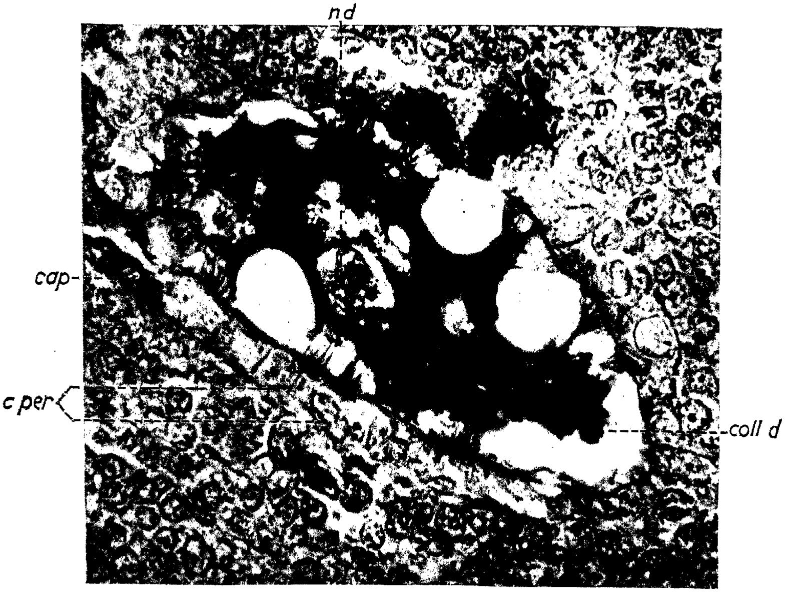

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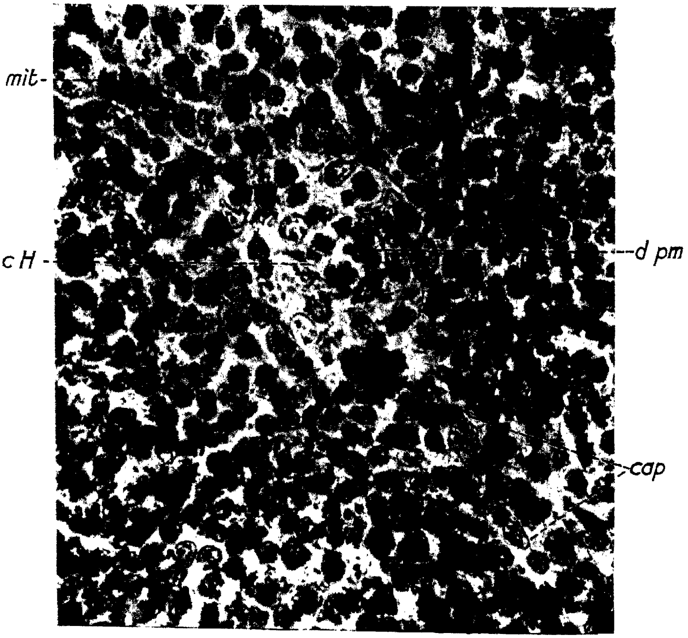

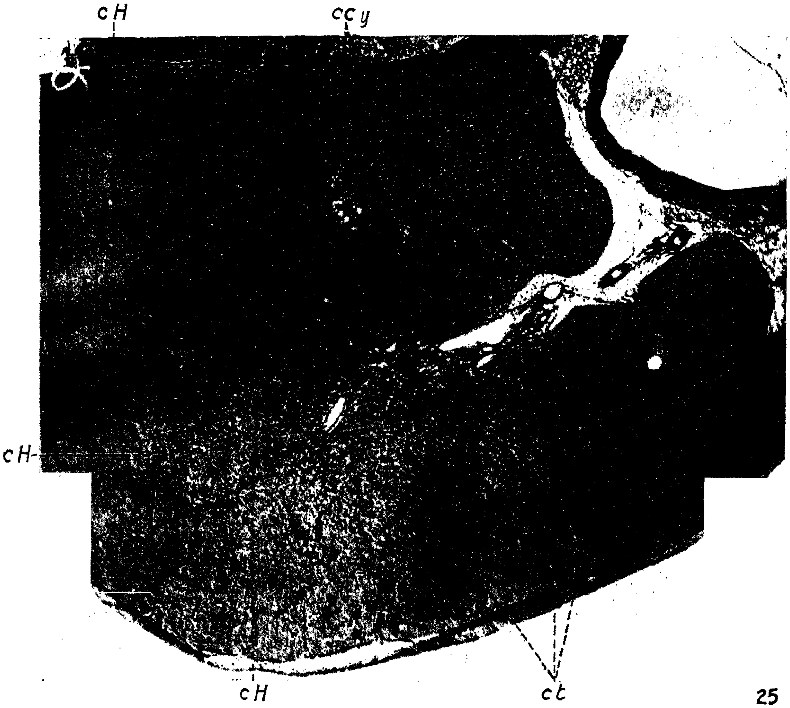

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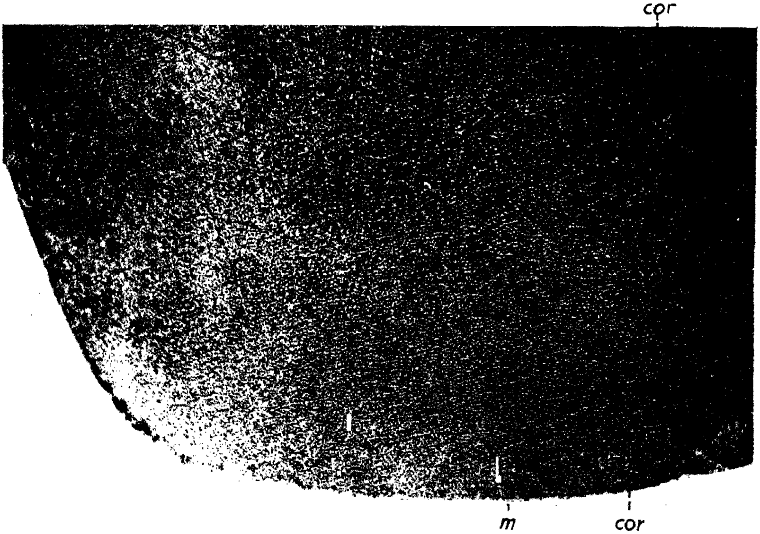

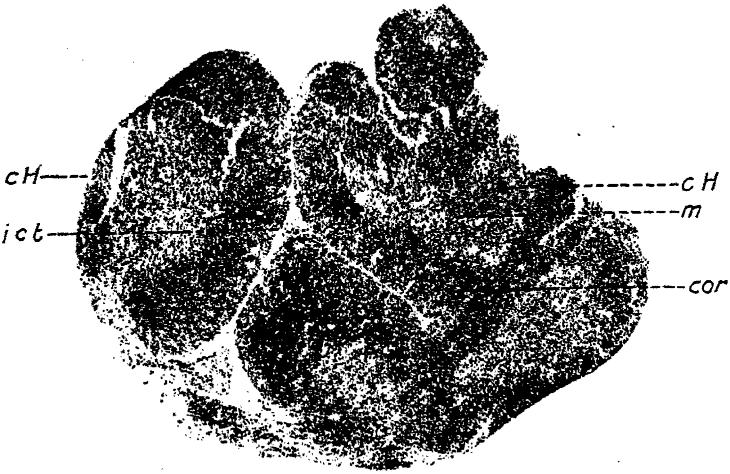

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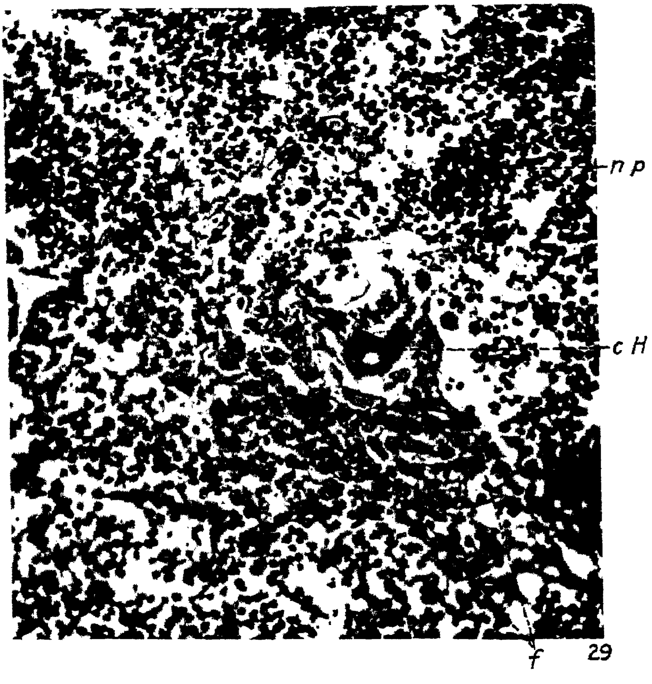

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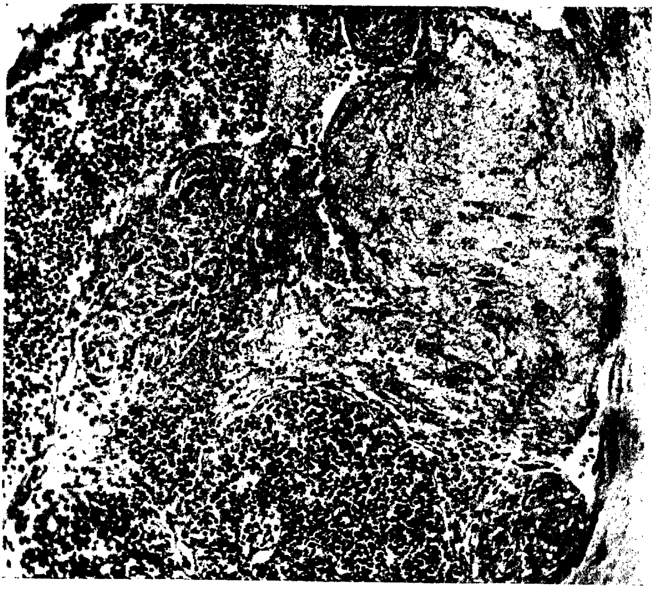

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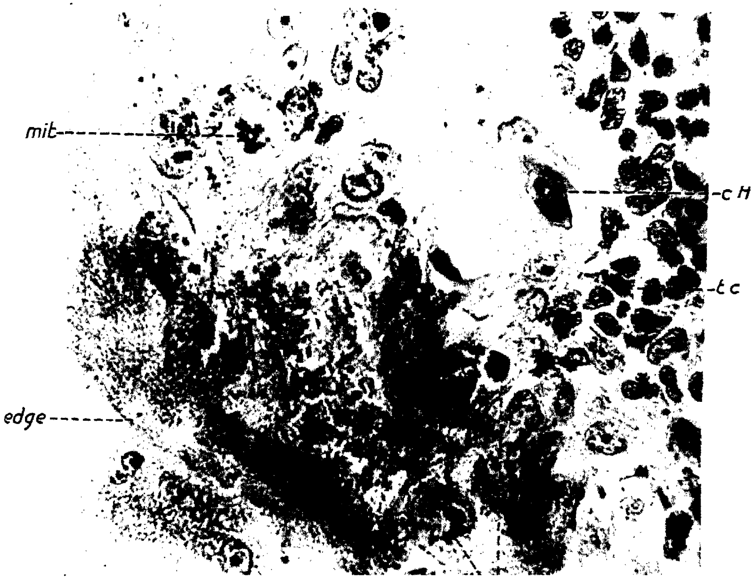

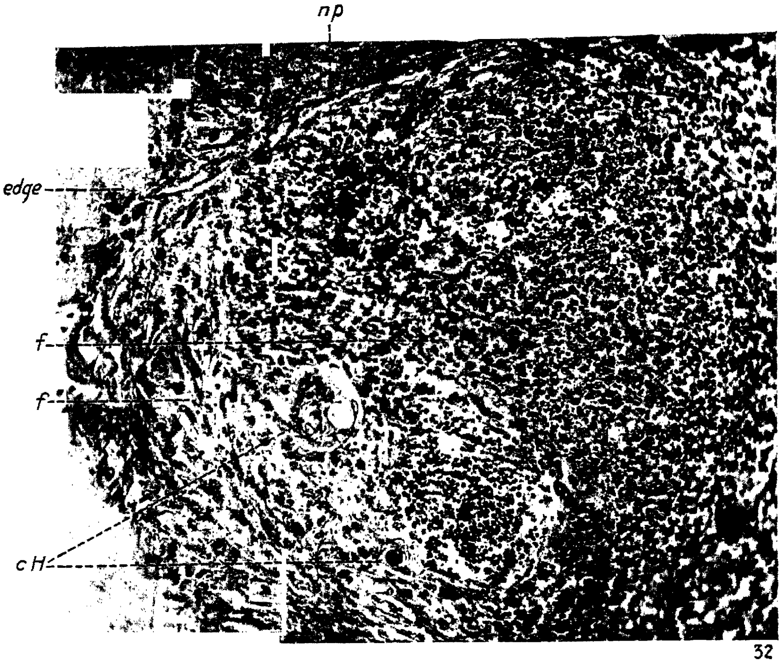

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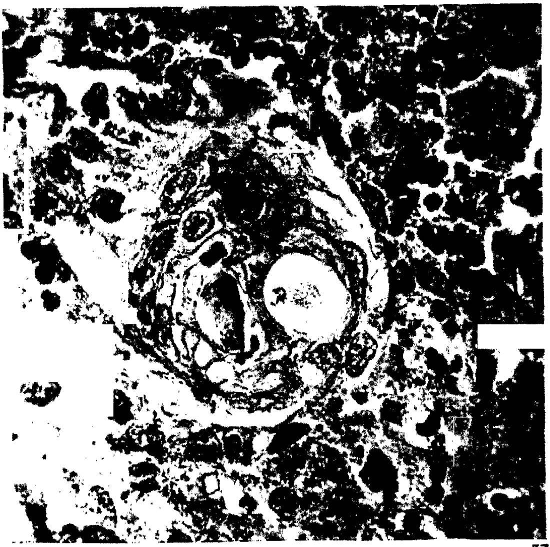

33

Studies in the Origin of Yolk.
I. Oogenesis of the Spider, Crossopriza lyoni (Blackwall).

By

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(Department of Zoology, Government College, University of the Punjab, Lahore.)

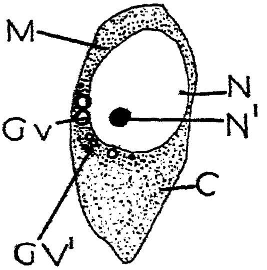
With 23 Text-figures.

INTRODUCTION.

In a previous paper (1924) I claimed that in Lithobius the granular Golgi elements of the young oocyte are transformed into fatty yolk-spheres which are quite distinct, both in their physical and chemical characteristics, from the albuminous yolk which probably originates from nucleolar extrusions of a remarkable type. In support of this claim it was stated that the fatty yolk appears only after the juxta-nuclear Golgi apparatus begins to fragment and spread out throughout the cytoplasm ; secondly, in Mann-Kopsch preparations all gradations exist between the Golgi elements and the fatty yolk ; and thirdly, experiments with the centrifuge most clearly show an increase in the fatty yolk and a corresponding decrease in the definite Golgi elements. A few similar claims had been made earlier than this. Ludford (1921) had confirmed the earlier observations of Gatenby and Woodger (1920), that in the egg of Patella the archoplasmic (idiosomic) substance of the Golgi batonette is loaded with fat and gives rise to fatty yolk. This claim has been further confirmed by Brambell (1924), who has also shown that in Helix aspersa the whole of the Golgi element, and not the archoplasmic sphere only as in Patella, gives rise to a fatty yolk-sphere. Gatenby (1922) had also described fatty yolk-granules in Saccocirrus which probably arose from the

Golgi elements. Hirschler (1917), building on the earlier work of Loyez (1909) on Ciona, Molgula, and Ascidia, had shown that in Ciona the Golgi elements become fatty and apply themselves to yolk-spheres which are mitochondrial in

TEXT-FIG. 1.

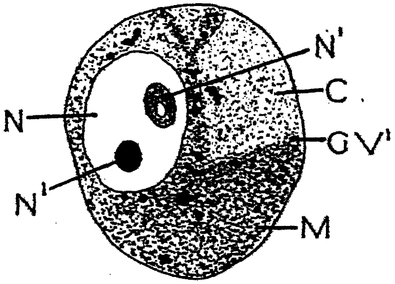


Mann-Kopsch unstained. × 1,050.

EXPLANATION OF LETTERING.

A.Y, albuminous yolk; C, cytoplasm; F.Y, fatty yolk; G.V, Golgi vacuole; G.V', Golgi vacuole looking solid on account of excessive osmication or staining; M, mitochondria; N, nucleus; N', nucleolus.

TEXT-FIG. 2.

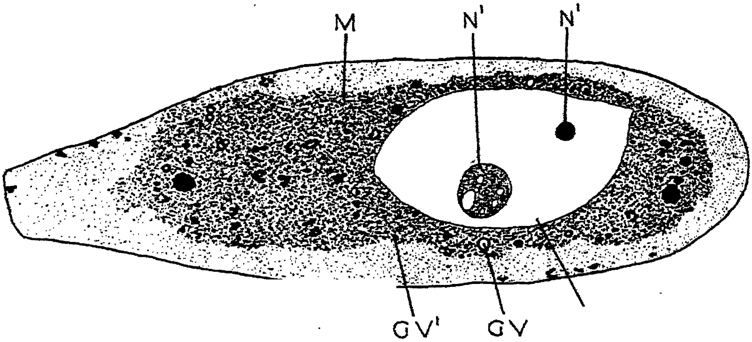


Mann-Kopsch unstained. × 1,050.

origin, thus giving rise to compound yolk-bodies. Most recently Harvey (1927) has confirmed the mitochondrial origin of yolk in Ciona intestinalis; but the fatty material of the yolk-spheres is not derived from the Golgi elements as claimed by Hirschler, but from the lipoidal granules of the test-cells, which material passes in solution into the cytoplasm of the oocyte.

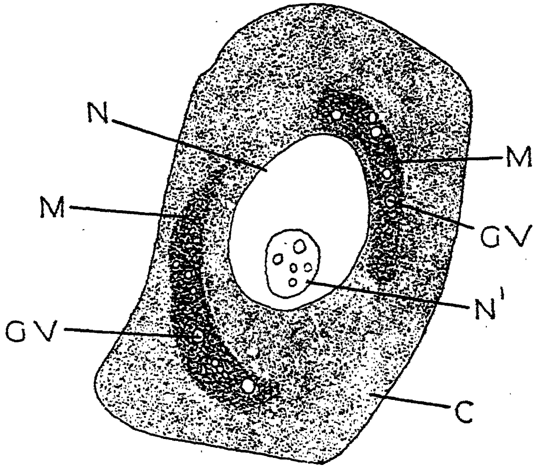
The Golgi elements, according to Harvey, secrete the 'yolk-nucleus' of an albuminous nature which fragments and disappears and contributes to yolk; but, according to Hirschler, the 'yolk-nucleus' is a purely cytoplasmic structure. It is, however, significant that Parat and Bhattacharya (1926), working with the vital dyes on the oocyte of Ciona, have confirmed

TEXT-FIG. 3.



Mann-Kopsch unstained. × 1,050.

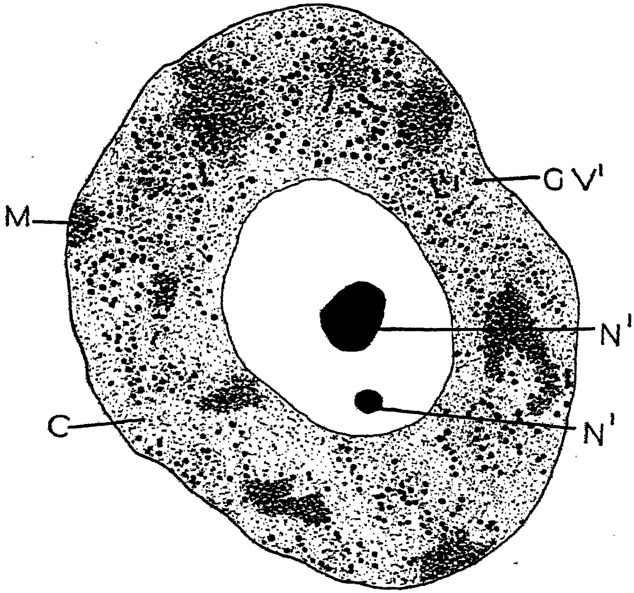
TEXT-FIG. 4.



Champy-Kull unstained. × 1,050.

Hirschler's details of the origin of yolk from the mitochondria and the Golgi elements, and consider that the 'yolk-nucleus' is an artifact. Recently Miss King (1926) has described most circumstantially that in Oniscus asellus the Golgi elements are directly metamorphosed into the fatty yolk-spheres by a process which is strictly comparable to that of Lithobius as described by Nath (1924), and to that of Helix aspera as described by Brambell (1924). The albuminous yolk is mitochondrial in nature.

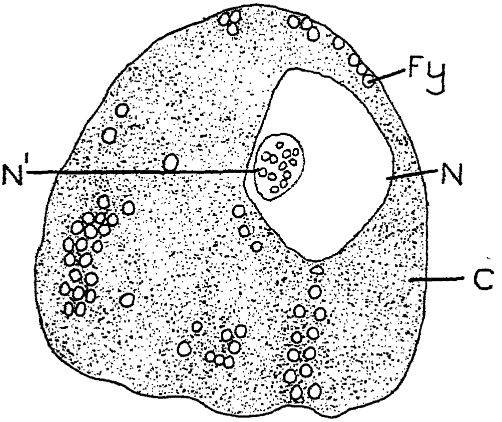
TEXT-FIG. 5.



Champy-Kull stained with iron haematoxylin. ×560.

These conclusions have received scant recognition and even denial from certain quarters, partly because of the inherent difficulties of such cytological work; but mainly because the supporters of the Golgi origin of the fatty yolk failed to prove any fundamental morphological resemblance between the Golgi elements and the fatty yolk-spheres—an evidence which alone could finally prove the claim. Since I published my paper on Lithobius it has been my endeavour to study the eggs of as many animals as possible, especially with the help of vital dyes, with a view to ascertain if there is any such resemblance. I believed that if one could show, in addition to the already strong arguments adduced in the case of Lithobius, &c., that these two types of inclusions in the cell are structurally similar with differences only in their size and chemical contents, the claim is proved to the hilt. In the course of my work I discovered that in Lithobius (1924), spider (1926 *a*), Scolopendra (1926 *d*), and the fire-fly Luciola gorhami (1927), the osmicated fatty yolk-spheres looked like clear vacuoles after they had been treated with turpentine or xylol. During the process of decolorization each osmicated fatty sphere showed a central chromophobic substance and a dull black chromophilic rim. Gradually the rim was also decolorized and the whole sphere looked like a clear vacuole. It was difficult, however, to ascertain the exact structure of the Golgi element. While I was studying some excellent Da Fano preparations of the Julus egg, I discovered that most of the Golgi elements were in the form of hollow spheres with a sharp black chromophilic rim and

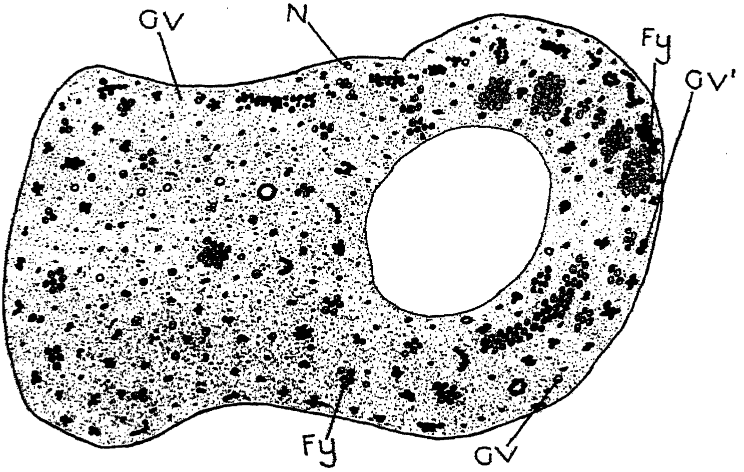
TEXT-FIG. 6.



Champy-Kull unstained. Kept in turpentine. × 630.

a central chromophobic substance. Some of them, however, appeared as solid spheres or as curved rods with the well-known archoplasmic or idiosomic substance. In the midst of this study I came across certain remarkable papers of Parat which showed clearly that the Golgi element is really a vacuole, and all other appearances are artifacts. Since I have already reviewed Parat's work in another place (1926 *c*), no more reference is necessary to his work in the present paper. When I attempted to ascertain the structure of the Golgi elements in the fresh eggs of spider, Scolopendra, Luciola, and Culex by the help of vital dyes, and compared it with those of the fixed eggs, I was struck by the remarkable accuracy of Parat's claims. I had already arrived at the definite conclusion that the fatty yolk-bodies are really vacuoles containing free fat, which after being oxidized by osmic acid makes these vacuoles look dull black and solid. Study of the fresh eggs of the above forms showed beyond all doubt the vacuoler nature of the Golgi elements, and also proved that in the eggs of spider, Scolopendra, and Luciola there are vacuoles of different sizes.

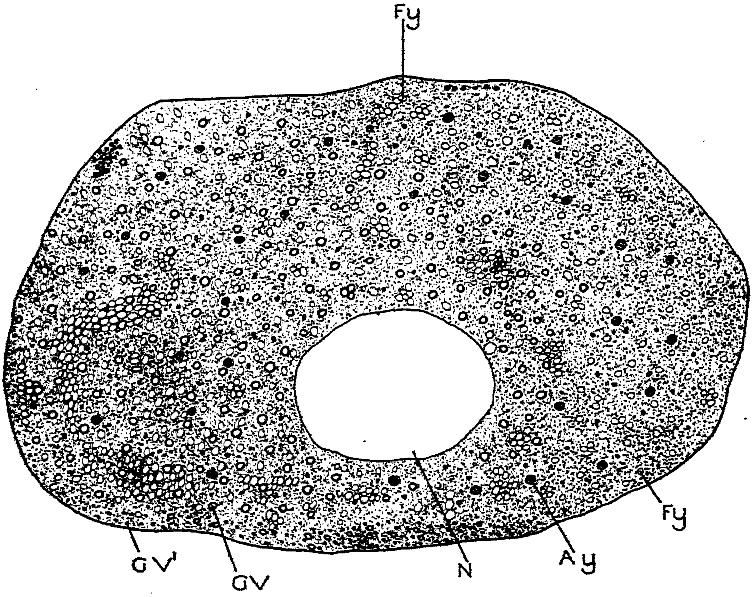
TEXT-FIG. 7.



Mann-Kopsch unstained. × 980.

Small ones are the Golgi vacuoles whose contents are watery, and big ones whose contents are fatty, as the fixed preparations clearly show, are the fatty yolk-vacuoles. Between these small and big vacuoles are vacuoles of intermediate dimensions. It thus became clear to me that the Golgi vacuoles by a process of growth and deposition inside their interior of colloids in the form of free fat not miscible with the general cytoplasm give rise to fatty yolk, and I announced this line of argument in 'Nature' (1926 *b*).

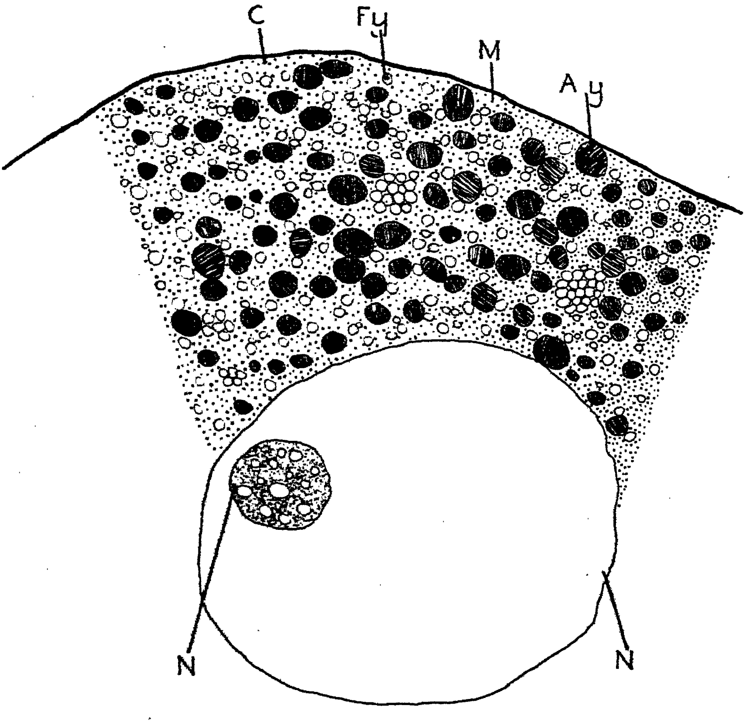
TEXT-FIG. 8.



Mann-Kopsch unstained. Kept in turpentine. × 980.

In addition to the routine technique I have employed the vital dyes, neutral red and janus green B. Very satisfactory results were obtained by the study of entire eggs after keeping them in 2 per cent. osmic acid from ten minutes to half an hour. Although osmic acid coagulates the protoplasm, it is a fixative that introduces the least change especially in the short period mentioned above. This is confirmed by a paper by Strangeways and Corti (1927), which has just reached my hands. These writers have shown that the cell after a short period of fixation with 2 per cent. osmic acid is almost like the living cell in vitro with regard to all the inclusions. The wall spiders on which this work is done were collected at Patiala and were identified by the authorities of the Indian Museum, to whom my thanks are also due for supplying me with the necessary literature.

TEXT-FIG. 9.



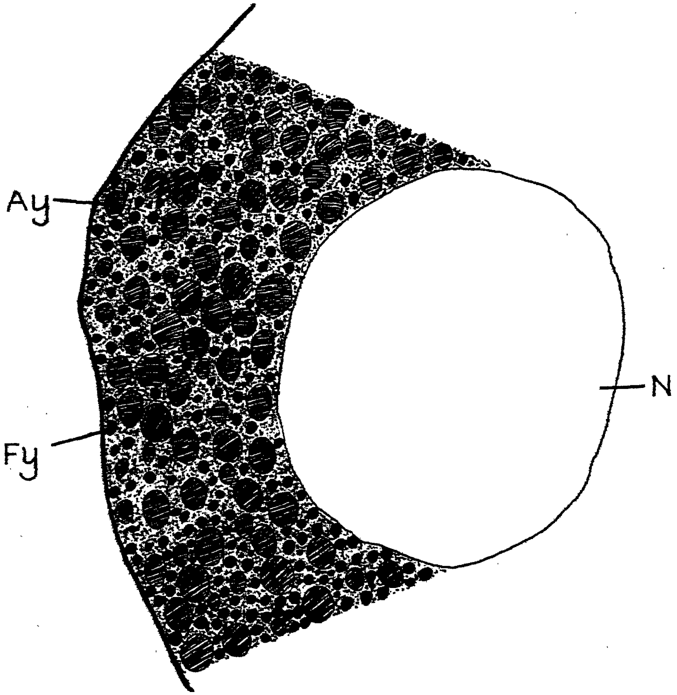
Champy-Kull and acid fuchsin. Kept in turpentine before staining. × 1,050.

It is a great pleasure to me to thank Colonel S. R. Christophers, F.R.S., for kindly giving me all facilities for this work in his institute at Kasauli in 1926.

PREVIOUS WORK.

Most of the work on the spider's egg has been carried out during the last century. Van Bambeke (1898), working on the spider Pholcus phalangioides, described in the young oocyte an almost complete circumnuclear ring, the pallial layer or the mantle layer (Wilson, 1925). In his excellent figures Van Bambeke shows the pallial layer consisting of fine granules, embedded in which are minute circular bodies. With the growth of the oocyte the pallial substance fragments throughout the cytoplasm, and gives rise to fatty deutoplasm which is blackened intensely by osmic acid and is decolorized quickly in turpentine or xylol. Nothing is said as to whether the granules or the

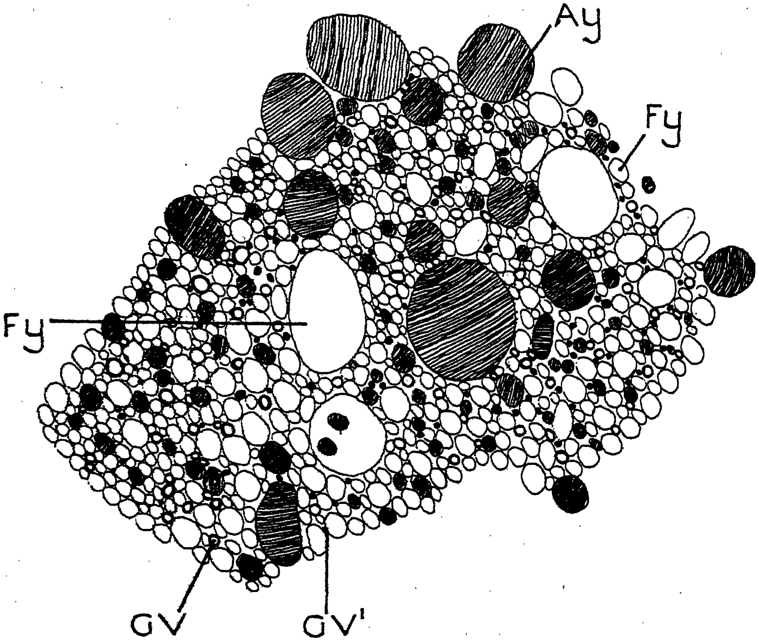
TEXT-FIG. 10.



Champy-Kull unstained. × 1,050.

circular bodies of the pallial substance give rise to the fatty yolk. On the contrary, although these constituents of the pallial substance are figured quite distinctly, they are not mentioned in the text. The albuminous yolk puts in its appearance de novo in the cytoplasm. It is indeed remarkable that in spite of the comparatively inefficient technique of those days Van Bambeke figured granules and circular bodies in his pallial substance which, as I have shown in the case of Crossopriza, are the mitochondria and the Golgi elements. Mitochondria had been discovered in those days, but they were very little known, and the Golgi apparatus was not yet even discovered. I have further shown that the Golgi elements only and not the whole pallial substance give rise to the fatty yolk, and I have also been able to confirm Van Bambeke's statement that the albuminous yolk arises independently in the cytoplasm.

TEXT-FIG. 11.



Mann-Kopsch unstained. Kept in turpentine. × 1,050.

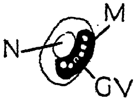
In another spider Tegenaria, however, as quoted by Wilson (1925), Wittich (1845), Carus (1850), Van der Stricht (1898), and Fauré-Fremiet (1910) have described a rounded body, the ' yolk-nucleus ' or the ' corps vitellin ', embedded in the finely granular pallial substance. Weiner (1925), as quoted by Harvey (1927), has also found a ' yolk-nucleus ' in Tegenaria, which is surrounded by the Golgi elements and is, in part at least, secreted by them. In Crossopriza, however, nothing comparable to the ' yolk-nucleus ' of Tegenaria has been discovered either in fixed or fresh material, and it may quite possibly be an artifact, as has been shown by Parat and Bhattacharya (1926), who have used vital dyes, in the case of Ciona, although Hirschler (1917) and Harvey (1927) believe it to be a true cytoplasmic structure. Nor has Van Bambeke described any structure comparable to the ' yolk-nucleus ' of Tegenaria in his own object, Pholcus.

OBSERVATIONS.

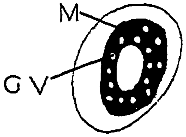
Golgi Elements and Fatty Yolk.

It has been possible to study all stages of the fresh oocyte with the help of neutral red or with neutral red and Janus green B used together or with 2 per cent. osmic acid only. In the youngest oocyte stained with neutral red the Golgi elements are seen in the form of a few colourless juxta-nuclear vacuoles lying very close to each other (Text-fig. 12). They are embedded in the mitochondrial mass which appears reddish and structureless on account of the precipitation of the stain in the spaces between the mitochondrial elements which are granular, as will very shortly appear. The cytoplasm is stained much less intensely. The study of the clear Golgi vacuoles is undoubtedly facilitated by the reddish background provided by the mitochondrial mass. With Janus green B, used either singly or with neutral red, the mitochondrial mass is green and the Golgi elements appear as colourless vacuoles. In the next stage the horseshoe-shaped cap, consisting of the mitochondria and the Golgi vacuoles, completely encircles the nucleus (Text-fig. 13).

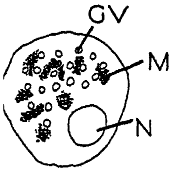
TEXT-FIG. 12.



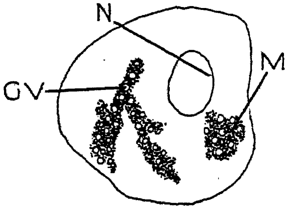
TEXT-FIG. 13.



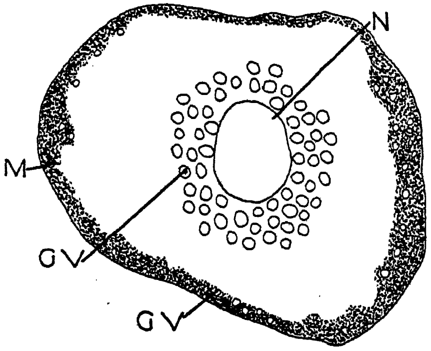
TEXT-FIG. 14.



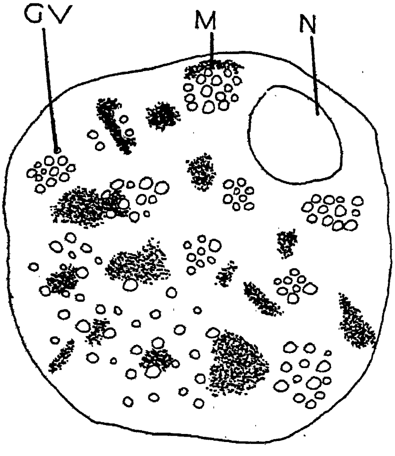
TEXT-FIG. 15.



TEXT-FIG. 16.



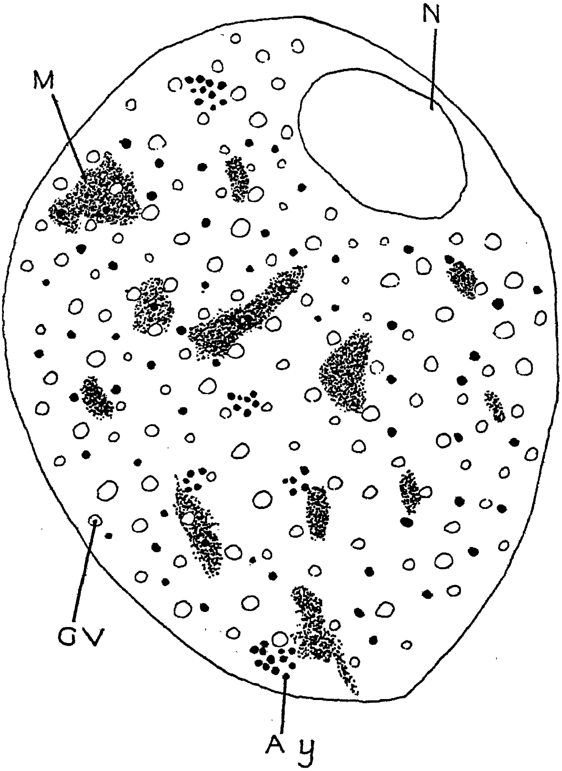
TEXT-FIG. 17.



Figs. 12–18. Fresh eggs stained with neutral red for about half an hour. All these figures are magnified about 270 times.

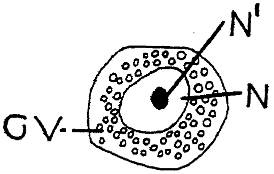
With the growth of the oocyte the mitochondrial granules and the Golgi vacuoles increase in number and spread out in the cytoplasm. Text-figs. 14–18 represent fresh eggs that have been stained with neutral red. The Golgi vacuoles increase in numbers rapidly, and many of them increase in size also. In the earlier stages (Text-figs. 14, 15, 16, and 17) they lie in groups, but by the time the albuminous yolk puts in its appearance (Text-fig. 18), they are more or less uniformly distributed throughout the cytoplasm. The contents of the bigger vacuoles are different chemically from those of the smaller ones, as will

TEXT-FIG. 18.

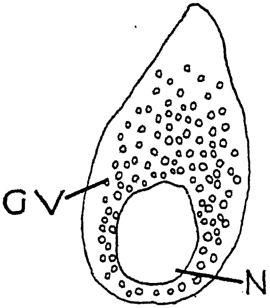


shortly appear, but in fresh eggs stained with neutral red they all look alike. I have failed to observe the Golgi vacuoles in the fresh egg after the stage represented in Text-fig. 18, for the reasons that in such an egg the increasingly thickening egg-membrane, the large number of albuminous yolk-spheres, and the increase in size of the egg itself do not allow sufficient amount of light to pass through it.

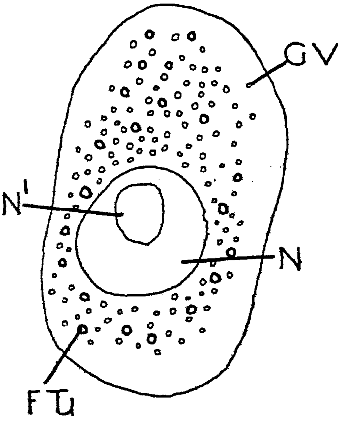
Text-fig. 19.



Text-fig. 20.



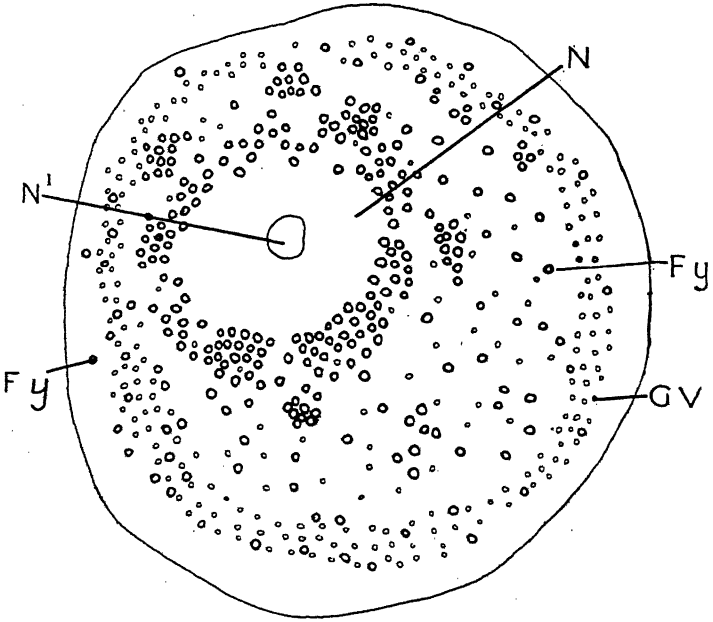
Text-fig. 21.



Figs. 19–22.—Fresh eggs kept in 2 per cent. osmic acid from 10 minutes to half a hour. All these figures are magnified 410 times.

Very satisfactory results were obtained when eggs were studied after a few minutes' immersion in 2 per cent. osmic acid. In such preparations it is possible to ascertain the chemical nature of the Golgi vacuoles, which is not possible either with neutral red or with Janus green B. Text-figs. 19–23 represent eggs kept in 2 per cent. osmic acid from ten minutes to half an hour. In Text-figs. 19 and 20 the Golgi elements completely surround the nucleus and appear as colourless vacuoles. The contents of these vacuoles seem to be watery and not fatty. In Text-fig. 21 some of the Golgi vacuoles have increased in size as shown at F.Y, and give rise to the fatty yolk. The fatty yolk vacuoles appear solid and black if the egg is kept in 2 per cent. osmic acid for more than half an hour; but if the period of osmication is reduced to about ten minutes, each fatty yolk-

TEXT-FIG. 22.



element appears as a vacuole with a black chromophilic rim and a central chromophobic substance. The contents of the smaller vacuoles shown at G.V. in Text-fig. 21 remain watery and non-fatty, and they continue to appear as colourless vacuoles. On account of their non-fatty and watery contents these vacuoles show a chromophilic rim and a central chromophobic substance only after a long period of osmication (14 days to 3 weeks), as will appear later. In Text-fig. 22 the number of fatty yolk-vacuoles has increased. They are more numerous round the nucleus (cf. Text-fig. 16). On account of the reasons already given the older eggs become opaque, and it is not possible to study them in whole mounts. The egg-membrane of older eggs, therefore, is ruptured by means of a needle, and its contents are allowed to flow out on the slide. Previous to this treatment the egg is kept in 2 per cent. osmic acid for one hour. Text-fig. 23 represents the contents of a ruptured egg. The albuminous yolk appears in the form of round, brownish, solid, and homogeneous spheres of different sizes. The Golgi elements appear as small colourless vacuoles, and the fatty yolk-vacuoles are blackened.

TEXT-FIG. 23.

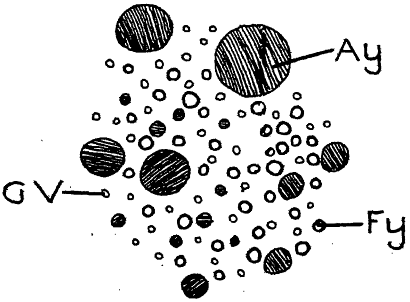


Fig. 23.—A portion of a crushed egg kept in 2 per cent. osmic acid for one hour. Big fatty yolk-vacuoles have burst and have disappeared. × 410.

Further explanation of figures will be found in the text.

It is clear that the Golgi vacuoles whose contents are watery and non-fatty give rise to the fatty yolk-vacuoles by a process of growth and deposition inside their interior of free fat not miscible with the general cytoplasm.

All the above observations are based on a study of whole mounts of fresh eggs either stained with the vital dyes or kept in 2 per cent. osmic acid for a short period. Such a study has enabled me to find out in fixed preparations what are natural structures and what are mere artifacts. Text-figs. 1, 2, 3, 4, 5, 6, and 7 represent various stages of the oocyte before the albuminous yolk puts in its appearance. Text-fig. 1 (Mann-Kopsch unstained) represents the youngest oocyte. The mitochondria appear as brownish granules forming a juxta-nuclear crescent in which lie the Golgi elements. The latter appear as vacuoles at G.V. with a sharp black rim and a central clear substance. At G.V^1, however, the whole of the Golgi vacuole is intensely osmicated and appears solid on account, undoubtedly, of the excessive precipitation of the metallic osmium not only on its surface but also inside its interior. In Text-fig. 2 (Mann-Kopsch unstained) all the Golgi vacuoles appear solid, and in certain places they form monilliform or irregular masses—appearances which are undoubtedly due to the excessive precipitation of metallic osmium not only on the surface and in the interior of these vacuoles but also in the spaces between them. In Text-figs. 3 and 7 (Mann-Kopsch unstained), in spite of the excessive reduction of osmium tetroxide, some of the Golgi elements appear as vacuoles with a sharp chromophilic rim and a central chromophobic substance. But Text-figs. 8 and 11 (Mann-Kopsch unstained) very faithfully show the vacuolar nature of the Golgi elements. Only a few look solid for reasons already given. The Golgi elements appear as vacuoles in other preparations also. Text-fig. 4 represents an unstained preparation fixed in Champy-Kull. The mitochondria appear yellowish in the form of two crescents which provide an excellent background for the clear Golgi vacuoles. Text-fig. 5 is a Champy-Kull preparation stained with iron haematoxylin. Here all the Golgi elements appear solid on account of the precipitation of the stain inside their interior.

The chemical nature of the contents of the Golgi vacuoles may now be described. It has already been mentioned that in fresh eggs stained with neutral red all the vacuoles which are of different sizes appear colourless. However, in fresh eggs after a short treatment with osmic acid one finds that there are two types of vacuoles: firstly, small ones whose contents are watery and non-fatty and which remain colourless, and secondly, bigger vacuoles whose contents are fatty and which, therefore, are blackened by osmic acid. In fixed preparations also one finds the same difference. The vacuoles with watery contents are generally smaller than those whose contents are fatty, although often vacuoles as small as those whose contents are watery may contain fat. The Mann-Kopsch and the Champy-Kull methods, in spite of the unjust criticism they have received from certain quarters, are valuable methods for the study of both types of Golgi vacuoles. In Text-fig. 8, which is an ideal Mann-Kopsch preparation, two types of vacuoles have been shown. At G.V. are vacuoles with a very sharp chromophilic rim and a central chromophobic substance. These are the vacuoles that undoubtedly contain a watery, non-fatty fluid, as is proved by the absence of a coagulum inside their interior. The second type of vacuoles shown at F.Y. do not show any sharply osmicated rim. They really appear solid and dull black in Mann-Kopsch preparations (Text-fig. 7, F.Y.), while the vacuoles with non-fatty contents, if over-osmicated, appear sharp black. The vacuoles with fatty contents are blackened by the osmic acid of the Mann-Kopsch method in a few minutes, while the non-fatty vacuoles take at least a fortnight to blacken. The fatty vacuoles when osmicated look solid. They can be decolorized by turpentine, when they appear as clear vacuoles as shown at F.Y. in Text-fig. 8. They are also decolorized if the slide is kept in xylol for a long time or if it is heated after mounting, which seems to accelerate the action of the balsam xylol on these bodies. Want of these precautions has led some workers to infer erroneously that there is no free fat in a cell. The solid appearance of the fatty vacuoles before treatment with turpentine or xylol points clearly to the existence of a coagulum, and their quick osmication and equally rapid decolorization to the existence of unsaturated fat which gives rise to the coagulum. It is thus clear that the Golgi vacuoles at first contain a watery non-fatty fluid, and gradually colloids in the form of free fat not miscible with the general cytoplasm are deposited inside their interior. It is undoubtedly these colloids that raise the refractive index of these vacuoles and enable us to study them in fresh eggs. Since in somatic and male germ-cells free fat is not as a rule elaborated in the cytoplasm, the refractive index of the Golgi vacuoles remains low. This accounts for the failure of so many attempts at studying the Golgi apparatus in such cells in the fresh condition, and for the remarkable ease with which such study is possible in the eggs of spiders.

The vacuoles that contain free fat give rise to fatty yolk by further deposition of free fat inside their interior and by an enormous increase in their size. Text-fig. 11 (Mann-Kopsch unstained) represents a portion of the most highly developed ovarian egg kept in turpentine for some time. At G.V. are the vacuoles with a sharp chromophilic rim and a central clear substance and containing a non-fatty fluid; at G.V.^{1}, are similar vacuoles looking sharp black and solid on account of the excessive precipitation of metallic osmium inside their interior; and at F.Y. are the fatty yolk-vacuoles which are dull black and solid before the slide is kept in turpentine. After decolorization they look like clear vacuoles which give a remarkable frothy appearance to the egg.

The Champy-Kull preparations both stained (either with iron haematoxylin or with acid fuchsin) and unstained as clearly show the vacuolar nature of the fatty yolk and Golgi elements as the Mann-Kopsch method. Text-fig. 6 represents an unstained Champy-Kull preparation kept in turpentine for some time. At F.Y. are shown certain vacuoles which are fatty, as is clearly proved by their quick osmication with osmic acid and equally quick decolorization in turpentine. The other vacuoles that contain a watery fluid cannot appear in this preparation unless it is stained, preferably with iron haematoxylin. In Text-fig. 4, however, which also is an unstained Champy-Kull preparation, Golgi vacuoles with watery contents do appear, on account of the very favourable yellowish background provided by the mitochondrial granules very closely aggregated together. The fatty vacuoles have not yet appeared in this oocyte as it is still very young. Similarly in Text-fig. 5 (Champy-Kull and iron haematoxylin) fatty vacuoles have not yet appeared, but the smaller vacuoles with watery contents are shown at G.V^1. They look solid on account of the precipitation of the stain inside their interior. Text-fig. 10 is a Champy-Kull unstained preparation of an advanced oocyte. At F.Y. are the osmicated fatty vacuoles that look solid and dull black on account of osmication. At F.Y. in Text-fig. 9, which is a similar preparation kept in turpentine and stained with acid fuchsin, are the fatty yolk-vacuoles.

A point of very great interest may now be mentioned. In my Da Fano preparations the Golgi elements appear as vacuoles with a sharp chromophilic rim and a central clear substance. Solid Golgi elements also appear on account of the excessive precipitation of metallic silver inside the interior of these vacuoles. The ' crescents ' also appear with the usual idiosomic substance. These ' crescents ' are entirely absent in fresh eggs and possibly are artifacts due to incomplete blackening of a vacuole. It seems, however, more probable that they are the optical sections of the vacuoles.

Mitochondria.

The breaking up of the mitochondrial mass, and the uniform distribution of the mitochondrial granules ultimately through the cytoplasm, are no less clear than the origin of the fatty yolk. Text-figs. 12–18 represent eggs stained with neutral red. In the youngest oocyte (Text-fig. 12) the mitochondrial mass appears solid and structureless on account of the very close aggregation of the mitochondrial granules and the precipitation of neutral red in the spaces between these granules. The same appearance is produced if the egg is stained with Janus green B, with the difference that the mitochondrial mass is green instead of red. The mass now grows in size and breaks up into smaller pieces that are scattered irregularly in the cytoplasm (Text-figs. 14, 15, 17, and 18). At the same time the mitochondrial granules loosen and in the mitochondrial patches granular mitochondria can be clearly seen either with neutral red or with Janus green B, but they are more brilliantly stained with the latter stain. Prior to the breaking up of the mitochondrial crescent the latter first grows into a complete circum-nuclear ring (Text-fig. 13), which later breaks up into smaller pieces. Fixed preparations show the structure and the dispersal of the mitochondrial granules as faithfully as the fresh ones. Text-figs. 1, 2, 3, 7, 8, and 11 are unstained Mann-Kopsch preparations. During the stages represented by Text-figs. 7, 8, and 11 the mitochondrial granules are uniformly distributed and, since these preparations are unstained, they do not appear. But in Text-figs. 1, 2, and 3, which are also unstained, they appear as brownish granules on account of their very close aggregation. Similarly in Text-fig. 4 (Champy-Kull) they can be seen as yellowish granules on account of their very close aggregation, in spite of the fact that this preparation is unstained. In Text-fig. 5 (Champy-Kull and iron haematoxylin) the mitochondrial granules can be seen in the act of dispersal. In Text-fig. 9 (Champy-Kull and acid fuchsin), which represents a portion of an advanced oocyte, the mitochondria are uniformly distributed and appear as small fuchsinophil granules.

Albuminous Yolk.

The albuminous yolk consists of round and homogeneous spheres which arise de novo in the cytoplasm. In fresh preparations stained with neutral red (Text-fig. 18) these spheres appear colourless and solid, while the Golgi elements and the fatty yolk appear as colourless vacuoles, and the mitochondria as fine red granules. In unstained Mann-Kopsch and Champy-Kull preparations they appear brownish or yellowish, while fatty yolk is black. They are stained pink with acid fuchsin and black with haematoxylin. The albuminous yolk-spheres are of different sizes (Text-fig. 9), but the youngest of them is very much bigger than a mitochondrial granule. They are perfectly preserved in Bouin's fluid, while the mitochondria, the Golgi elements, and the fatty yolk are destroyed.

Centrifuging.

Experiments with the centrifuge gave results identical with those in the case of Lithobius (Nath, 1924). The eggs were centrifuged for about half an hour in normal saline with a hand centrifuge. They were then fixed with the Champy-Kull method and stained with acid fuchsin. The albuminous yolk occupies one pole and is stained pink. The opposite pole is occupied by the fatty yolk-bodies that appear solid and black. The central area contains numerous small fuchsinophil granules which are the mitochondria, as also the nucleus which is often pushed slightly into the fatty yolk area.

Nucleolus.

The nucleolus in the youngest oocyte is a solid rounded body. It is highly basiphil. With the growth of the oocyte it increases in size, develops vacuoles inside it, and becomes acidophil. It may bud off another nucleolus from it (Text-figs, 2, 3, and 5). In fresh preparations also it appears as solid body (Text-figs. 19, 21, and 22). There are no nucleolar extrusions.

SUMMARY.

1. The oogenesis of the spider Crossopriza has been worked out by fresh cover-slip preparations stained with neutral red and Janus green B or kept in 2 per cent. osmic acid from ten minutes to half an hour. Routine laboratory methods have also been used.

2. Treatment with 2 per cent. osmic acid for the period mentioned above does not introduce any artifacts.

3. In the youngest oocyte the Golgi elements are in the form of vacuoles containing a watery and non-fatty fluid, and are embedded in the mitochondrial mass.

4. The solid granular Golgi elements are artifacts produced by the excessive precipitation of metallic silver or osmium inside the vacuoles.

5. The crescent-shaped Golgi elements are also artifacts produced possibly by the incomplete blackening of vacuoles. More probably, however, the crescents are the optical sections of the vacuoles.

6. A process of growth and deposition of fat not miscible with the general cytoplasm inside the Golgi vacuoles gives rise to the fatty yolk-vacuoles.

7. The mitochondria are granular and form a horse-shoe-shaped cap on one side of the nucleus of the youngest oocyte. The cap gradually grows into a complete circum-nuclear ring. The ring breaks up, and ultimately the mitochondria are distributed uniformly throughout the cytoplasm.

8. There are no nucleolar extrusions. The albuminous yolk arises independently in the cytoplasm.

9. Experiments with the centrifuge have been performed.

10. The earlier literature on the origin of fatty yolk has been reviewed.

11. There is no structure in the egg of Crossopriza comparable to the 'yolk-nucleus' of the spider Tegenaria described by earlier writers.

BIBLIOGRAPHY.

1. Brambell, F. W. Rogers (1924).—'The British Journal of Experimental Biology', vol. i.
2. Fauré-Fremiet (1910).—'Archives de l'anatomie microscopique' (Paris), vol. ii.
3. Gatenby, J. Brontë (1922).—'Quart. Journ. Micr. Sci.', vol. 66.
4. Gatenby and Woodger (1920).—'Journ. Roy. Micr. Soc.'
5. Harvey, L. A. (1927).—'Proc. Roy. Soc. Lond.', Series B.
6. Hirschler, J. (1917).—'Arch. Mikr. Anat.', vol. 89.
7. King, S. D. (1926).—'Proc. Roy. Soc. Lond.', Series B.
8. Loyez, M. (1909).—'C. R. Assoc. Anat.'
9. Ludford, R. J. (1921).—'Journ. Roy. Micr. Soc.'
10. Nath, V. (1924).—'Proc. Camb. Phil. Soc. Biol. Sci.', vol. i.

11. Nath, V. (1926 *a*).—‘ Nature ’, May 15.
12. —— (1926 *b*).—Ibid., November 27.
13. —— (1926 *c*).—‘ Biol. Reviews, Camb.’, vol. ii.
14. Nath and Husain (1926).—‘ Nature ’, November 6.
15. Nath and Mehta (1927).—Ibid., January 1.
16. Parat, M., and Bhattacharya, D. R. (1926).—‘ C. R. Soc. Biol.’, vol. 94.
17. Strangeways, T. S. P., and Canti, R. G. (1927).—‘ Quart. Journ. Micr. Sci.’, vol. 71.
18. Van Bambeke (1898).—‘ Arch. de Biol.’, tome 15.
19. Van der Stricht (1898).—‘ Verhandlungen der Anatomischen Gesellschaft ’, vol. 12.
20. Weiner, P. (1925).—‘ Arch. Russ. Anat. Hist. Embr.’, vol. 4.
21. Wilson, E. B. (1925).—‘ The Cell.’

Yolk-Absorption in Loligo and the function of the embryonic liver and pancreas.

By

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With Plates 13–19.

Although many details of Cephalopod development have long been known to biologists, the physiology of the developing animal was, for many years, practically unstudied. The method by which the yolk is absorbed was, in particular, completely unknown. Faussek, in his paper of 1901, summarized the knowledge then existing, and so few have been the additions of later years that, in the brief abstract now given, we follow him almost verbatim.

In Loligo, as in Cephalopoda in general, the yolk is enclosed in a sac known as the vitelline membrane ('Dotterhülle', 'Dotterepithel', &c.). No separate cells are visible in this membrane, but large nuclei are scattered irregularly throughout the cytoplasm. The yolk-sac is divided, like an hour-glass, into two parts, an inner, and an outer (fig. 1, Pl. 13) : the whole is entirely closed, and has no communication with any other organ of the body (fig. 3, Pl. 15). During development not only is the yolk assimilated by the embryo, but, from a stage a little earlier than that represented in fig. 1 *a*, Pl. 13, yolk passes continuously from the outer into the inner yolk-sac. This transference takes place more rapidly than the assimilation of the yolk inside the animal, with the result that, as development proceeds, the outer yolk-sac grows smaller, the inner yolk-sac larger, and, by its growth, all surrounding organs are pressed against the body-wall.

Korschelt and Heider (1893), arguing from its very intimate relationship with the yolk-sac, supposed that the mid-gut was the yolk-absorbing organ. As Faussek points out, however, the cells of the mid-gut remain inactive and embryonic in character up to the time of hatching; nor is the surface offered by it sufficient to permit of the necessary volume being absorbed at a probable rate.

On the other hand the outer yolk-sac is entirely surrounded by a blood-space, which lies between the vitelline membrane and ectodermal covering of the organ (figs. 1 and 3, Pls. 13 and 15); and it was pointed out by Bobretzky (1877) that those contractions of the outer yolk-sac already observed by Lankester, and thought by him to drive the yolk from the outer to the inner yolk-sac, would, in fact, have the effect of keeping the blood surrounding the yolk-sac in continuous movement. Moreover, not only is the outer yolk-sac bathed in blood, but the inner yolk-sac is almost entirely surrounded by, or suspended in, a blood-space. These facts, taken together, led Faussek to the conclusion that 'without doubt the food material is withdrawn from the yolk by the blood'.[1]

Recently one of us (Portmann, 1926) made a series of experiments, on Loligo vulgaris, which definitely refuted the hypothesis of Korschelt and Heider. Observations made on the living embryo, and confirmed by histological examination, not only entirely supported the views of Bobretzky and Faussek, but revealed a complete embryonic blood-circulation between the embryo and outer yolk-sac, effecting absorption of the yolk in a manner comparable to that in the vertebrate embryo.

This embryonic circulation persists only so far as the stage represented in fig. 1 *a*, *b*, Pl. 13. At this stage the growth of the arm musculature has reached such a point that there is no longer room for the blood to pass between the embryo and outer yolk-sac, and the circulation of the outer yolk-sac is thus interrupted. At the same time the yolk begins to pass from the outer to the inner yolk-sac: not, as was previously thought, by contraction of the outer yolk-sac itself, but by regular contractions of the circumoral musculature, which begin at this period and continue during the whole period of growth of the inner yolk-sac.

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[1] 'Unzweifelhaft wird das Nährmaterial aus dem Dotter vom Blut aufgenommen', loc. cit., p. 147.

By this process the inner yolk-sac becomes more and more elongated, and the embryo is correspondingly stretched out round it. At the same time, the blood sinus surrounding the internal yolk-sac is compressed by the continual growth of the latter, until its anterior part is first reduced to a network of small vessels, and finally disappears, while the posterior part persists as the abdominal vein. Thus the mechanism which, for a time, transferred nutritive material from the yolk-sac to the organs of the body, is entirely destroyed.

The detailed observations of Portmann extended only to the break-down of the circulation of the outer yolk-sac. A brief examination of sections of more advanced stages was sufficient, however, to show, on the one hand, considerable growth of and signs of activity in the liver, and, on the other hand, very close topographical relations between that organ and the internal yolk-sac. It was suggested in his paper that the liver might take part in the absorption of the yolk during this last phase, and the work of which we here give an account was started in the endeavour to pursue this suggestion to some conclusion.

From our investigations it appears that the time of yolk-absorption in Loligo is divided into four periods, each of which is marked by important physiological changes in the developing animal. These periods are defined below.

In this definition, as in the subsequent description and discussion, the following terminology is employed :

(1) The orientation of the body is taken from that of the swimming animal, following the convention established by Naef in his monograph (1921, 1923). The arms are anterior, the fins posterior, the shell is dorsal or superior, the funnel ventral or inferior.

(2) The eighteen arbitrary ' stages ', into which Naef divides the embryonic life up to hatching, are used to indicate the age of the embryos described.

(3) The terms ' embryo ' and ' larva ' are employed respectively to distinguish the unhatched and hatched animal.

The four periods of yolk-absorption are:

Period I. From the first segmentation to the first contractions of the wall of the outer yolk-sac by which the embryonic blood-circulation is established. (Stages I–IX of Naef.)

Period II. From the first contractions of the wall of the outer yolk-sac to the point at which the blood-circulation between the embryo and outer yolk-sac is interrupted by growth of the circumoral musculature. (Stages IX–XVI of Naef.)

Period III. From the interruption of the circulation between the embryo and the outer yolk-sac to the complete emptying of the outer yolk-sac. Hatching may occur either at the end, or just before the end of this period. In the latter case the external yolk-sac often breaks off before its contents have passed completely into the inner yolk-sac. (Stage XVI of Naef to hatching.)

Period IV. From the emptying or casting off of the outer yolk-sac up to the complete absorption of the yolk. During the whole of this period the animal is a free-swimming larva.

Species of Loligo were chosen by us from among the Cephalopoda, as the young of this genus are completely transparent, so that it is possible to control histological work by observations on the living animal. Our material consisted of embryos of Loligo vulgaris from Banyuls-sur-mer, and of Loligo media from Plymouth. The material fixed at Banyuls consisted entirely of unhatched embryos; the specimens obtained at Plymouth were almost all larvae which had been hatched out in captivity. The cytological observations have all been made from sections of fixed material; the morphological observations were made partly on the living embryo.[1]

This paper discusses that part of the life-history of Loligo which is contained in our Periods III and IV.

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[1] As fixatives we used Bouin's fluid, the mixtures of Zenker and Helly, and Flemming's solution without acetic acid (strong formula). Helly's mixture (Zenker-formol) proved an excellent fixative for liver and vitelline membrane, but not for pancreas. For all histological technique we have followed Langeron (1925).

PERIOD III: THE GROWING OF THE INTERNAL YOLK-SAC.

Initial Conditions.

The general appearance of the embryo at the beginning of Period III is given in fig. 1 *a*, *b*, Pl. 13, and fig. 5 *a*, *b*, Pl. 16. A certain amount of yolk has already been driven into the embryo, which is now as long as, or slightly longer than, the outer yolk-sac. By far the greater volume of yolk is still in the outer yolk-sac, but the inner yolk-sac now extends into the posterior part of the body. It consists essentially of three parts, an anterior and two posterior lobes, the former being connected with the external yolk-sac by a narrow neck of yolk which passes through the brain.

It will be seen from the figures that the anterior lobe of the inner yolk-sac is limited in front by the brain, the statocysts, and, more dorsally, by the posterior salivary gland, while, posteriorly, it is bounded by the heart, gill-hearts, stomach, caecum, &c., packed closely together in one mass. At the point where the oesophagus turns down to meet this 'visceral mass', the yolk divides on either side of it and extends into the posterior part of the body as the two posterior lobes, closely applied to one another, and each connected with the anterior lobe. (See fig. 1, Pl. 13; fig. 2, Pl. 14; figs. 3–4, Pl. 15, and the transverse sections shown in fig. 5 *a* and *b*, Pl. 16.)

The blood-sinus which surrounds the internal yolk-sac during Period II has now been almost squeezed out of existence. The portion surrounding the posterior lobes, which is called by Faussek the 'posterior sinus', persists, indeed, as a narrow, blood-filled space, enclosed between the vitelline membrane and a tenuous epithelium, and ultimately becomes the 'abdominal vein' of the adult; but the sinus which previously surrounded the anterior lobe is now reduced to a mere network of small spaces, interspersed with mesoderm cells.

The yolk appears, in section, broken up into globules or plates. It stains readily with orange G., eosin, indigocarmine, bleu de Lyon, and lichtgrün. Of the nature of the living yolk almost nothing is known; since all our methods were selected for the study of the liver and vitelline membrane, and did not prove suitable for an examination of the yolk. We hope to carry out further investigations in this matter.

The vitelline membrane is remarkable at this period for its great thinness. Towards the end of Period II the vitelline membrane of the internal yolk-sac appears in section as an uneven line or band, staining rather strongly with haemalum. At the moment under consideration, however, the beginning of Period III, the vitelline membrane is so thin that it is often impossible to make it out as a continuous sheet. It stains very lightly with haemalum, and, in one preparation, stained in borax carmine, Bismarck brown, and bleu de Lyon, it even stains a brilliant blue, like the yolk itself. The nuclei, which during Period II were subspherical or ellipsoidal, are usually at this period flattened discs, drawn out, as it were, in the thin epithelium. It may also be mentioned here that, as no yolk-globules or droplets have ever been observed in the blood-stream, the yolk must be completely changed by the activity of the vitelline membrane. Immediately within the vitelline membrane a layer is often present which stains differently from the normal yolk. We have assumed that this layer represents the products of the activity of the vitelline membrane, and describe it as 'transformed yolk'. Although present both in earlier and later stages of development, during the beginning of Period III, no such layer appears.

The thinness of the vitelline membrane is easily explained by supposing that the continual inflow of yolk into the inner yolk-sac stretches the surrounding membrane as if it were the skin of an inflating balloon. The absence of accumulated transformed yolk may be due either to a low rate of activity on the part of the vitelline membrane, or to a rapid rate of absorption by the embryo. The external yolk-sac being no longer available as an organ of absorption, the embryo may well be in great need of food, and so take up rapidly all that is prepared. That the condition of the vitelline membrane at this phase is different from that of any other period is shown by its unusual resistance to nuclear stains.

Before describing the liver at the beginning of Period III it is necessary to give a brief account of its early development.

The rudiments of the 'hepato-pancreas' arise early in Period II (during X–XII of Naef) as a pair of lateral, finger-like diverticula, given off from the junction of fore- and mid-gut. These processes are directed anteriorly and project into the blood-sinus which, at this stage, surrounds the internal yolk-sac. They are closely surrounded by two or three layers of mesoderm cells (fig. 13, Pl. 19). The lumen of these tubes is probably, in life, exceedingly small, or almost non-existent; the wall is a simple columnar epithelium, showing many cells in mitotic division. As the inner yolk-sac grows so also does the hepato-pancreas; it grows forward between the yolk-sac and the body-wall, and also in a dorso-ventral direction. The presence and growth of the yolk-sac prevents much lateral expansion, so that the anterior part of the organ becomes elliptical in cross-section instead of circular (fig. 5 *a*, Pl. 16). Its posterior part is, however, unrestricted by the yolk-sac and expands in all directions, opening by a wide mouth into the rudiment of the caecum. The anterior part of the organ, which lies alongside the yolk-sac, will become the so-called 'liver' of the adult: the posterior part, which lies behind the yolk-sac, will become the 'pancreas'; 'liver' and 'pancreas' are connected by a narrow channel.

At the beginning of Period III the disappearance of the intervening blood-space has brought the liver and yolk-sac into fairly close relationship, but the two or three layers of mesoderm cells which surround the former organ still intervene between them. The liver cells are still embryonic in character with a high nucleo-cytoplasmic ratio (fig. 7, Pl. 17), but the first traces of secretion are now visible both in the cavity and accumulated in the distal or free ends of the cells. It is noteworthy that at this same stage the first signs of activity appear in the ink-gland, while the posterior salivary gland is already very active.

The conditions of liver and yolk-sac at this time have been described in some detail, as they are of considerable importance, representing a state of great change and of transition from the absorption of the yolk by blood alone to a totally different process.

Changes during Period III.

During Period III the inner yolk-sac grows steadily and, at the end of the period, has reached its maximum size. The vitelline membrane thickens rapidly, and once more stains readily with haemalum and iron haematoxylin, while liquefied yolk appears in increasing amount. In short, it would seem that the vitelline membrane is now growing as fast as the inner yolk-sac, and yolk is being converted more quickly than the embryo can absorb it.

The liver grows steadily in length and depth and becomes divided into a system of tubules, while the secretion in the liver increases in quantity. The most important change, however, is that the mesoderm cells which separated liver and yolk-sac have now largely disappeared, so that the vitelline membrane and liver-cells are directly, often closely, in contact, with only a few small, scattered cells appearing between them. During this period the blood-spaces round the anterior lobe diminish continually, while, on the other hand, a regular blood-supply to and from the liver develops, definite vessels become visible running from the aorta to the liver and from the liver to the vena cava, and the pancreatic sinus is formed.

Conditions at the End of Period III.

At the end of Period III the internal yolk-sac assumes the shape and dimensions indicated in fig. 1 *c*, *d*, Pl. 13, and fig. 2 *a*, Pl. 14, and occupies almost the whole of the body-cavity behind the brain and statocysts. The visceral mass is confined within the narrow space between the anterior and the two posterior lobes which project into and fill the pericardium almost completely. Accumulations of transformed yolk are present in such places as the peripheral regions of the posterior lobes, and the posterior part of the anterior lobe, where the curvature of the surface of the yolk-sac is high: as if the unabsorbed fluid collected in the hollows of the inner surface of the vitelline membrane. From this stage onwards it becomes increasingly difficult to draw a definite line between vitelline membrane and transformed yolk. With different methods of fixation and staining such very different pictures are produced that it is not possible to visualize a single set of conditions which could give rise to all. The impression received by the observer is, on the contrary, one of a state of constant change and chemical activity. Although it may be assumed that the activity or activities are rhythmic in character, it has not been possible, from the material examined and the methods employed, to obtain any light on the nature of the processes taking place. One fact, however, becomes clear: that part of the vitelline membrane which is in contact with the liver is different from the rest. This difference is not always obvious, and, when visible, takes varying forms: the importance of these facts in relation to the problem under consideration needs no emphasis.[1]

By the end of Period III the liver has grown forward until it overlaps the statocysts; it is now in the form of two very thin, wide lobes, closely applied to and practically covering the two sides of the anterior lobe of the inner yolk-sac (fig. 1 *d*, Pl. 13; fig. 2 *a*, Pl. 14). In a transverse section through the anterior lobe that part of the vitelline membrane which is opposite the liver is about one-third of the total circumference of the lobe, as compared with one-sixth at the beginning of Period III (cf. fig. 5 *a*, Pl. 16, and fig. 14, Pl. 19). The contact between the liver and vitelline membrane is very intimate, intervening cells are only sparsely scattered (fig. 8, Pl. 17), and it is sometimes even difficult to determine where the substance of the liver ends and that of the vitelline membrane begins.

The walls of the tubules are generally one, sometimes two, cells thick; the nuclei of the liver cells are smaller than at the beginning of Period III, and the nucleo-cytoplasmic ratio has proportionally decreased. In this diminution in size the nuclei of the liver cells present a strong contrast to those of the vitelline membrane, which have, if anything, increased in size, and which are now, in their greatest diameter, between two and three times as large as the nuclei of the liver cells.

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[1] In the preparation shown in fig. 11, Pl. 18, it appears clear that the difference, marked as it is, is one of degree only; the granules appearing in such large quantities opposite the liver are also present, though in smaller numbers, in the rest of the vitelline membrane, and are everywhere concentrated near the outer surface of the membrane.

These are, in brief, the conditions to be found in the liver and yolk until the end of Period III, a short time before hatching. What light do they throw upon the manner of yolk-absorption during this period ?

The fact that accumulations of transformed yolk are present suggests that, during this period, the absorption is not very rapid. It is certain that some absorption is taking place through the sinus surrounding the posterior lobes, and some through the much reduced and ever diminishing blood-system of the anterior lobe : are these the only absorbing organs ? We would recall three facts which have been cited : first, a very close contact between liver and yolk-sac ; second, a commencement of activity in the liver ; third, a differentiation of the vitelline membrane where it is in contact with the liver. These, taken together, point strongly to a physiological relationship between the yolk-sac and liver, but are hardly sufficient alone to prove that the liver is actually absorbing the yolk. It is to the fourth period of yolk-absorption that we must turn for definite evidence on this question.

PERIOD IV : THE DISAPPEARANCE OF THE INTERNAL YOLK-SAC.

This final period of yolk-absorption takes place under conditions very different from those prevalent in the three earlier periods : not only is the animal now leading an active, free-swimming life in or near the surface waters, but it is probable that, soon after hatching, it begins to feed, so that the yolk ceases to be its only source of nourishment. No direct evidence of this has been obtained (see Appendix A), but the jaws and radula of the animal are chitinized shortly after, if not before, hatching ; while the stomach and caecum show a functional aspect for the first time, and increase in size as the yolk diminishes. The larvae hatched in captivity from which the bulk of our material was obtained all died after a week ; the yolk-sac was then empty and death was presumably due to starvation. Thus many of the larvae used by us for this work were probably in a half-starving condition, a fact which, although important, does not alter the main argument of our hypothesis.

As Period III was a period of growth of the inner yolk-sac, so Period IV is the period of its diminution. Before all the yolk is actually absorbed the yolk-sac becomes invisible in the living larva, since it is entirely enclosed in the liver except where it is covered in front by the posterior salivary gland. For, whereas in Period III the continual inflow of yolk made the inner yolk-sac the dominant organ of the body, in Period IV the growth of the liver gives to this organ a more and more important position. This growth is not outwards but inwards ; that is to say, as the yolk-sac disappears it is in fact gradually replaced by the liver, as was observed by Lankester (1875). This process is illustrated clearly in fig. 2, Pl. 14, and figs. 5, 6, Pl. 16, which show also how, as the liver encroaches more and more upon it, the yolk-sac loses definite form, fitting into and filling the irregular space which remains between the two liver lobes. Thus the yolk-sac, which began its existence almost entirely surrounded by blood, ends it almost entirely surrounded by liver, and the conclusion suggests itself to the observer that the function performed at first by the blood is later fulfilled by the liver. A detailed examination of the histology of the two organs confirms this hypothesis.

Muscular and Secretory Functions of the Vitelline Membrane.

During Period IV the vitelline membrane shows considerable changes. The first and most remarkable of these is the formation of muscle-fibres making a regular muscular system for the inner yolk-sac. The existence of such fibres has never before been observed, as no histological research has been made hitherto on these late stages; their appearance is especially interesting since the syncytium which forms the vitelline membrane has always been regarded as a purely digestive organ. Our observations show that it combines digestive and muscular activities.

The muscle-fibres lie in the vitelline membrane immediately next the yolk, to the surface of which they are everywhere tangential (fig. 12 *a* and *b*, Pl. 18); the maximum length of a single fibre measured by us is about 20μ. The fibres do not develop simultaneously or equally throughout the yolk-sac. In a larva hatched a few hours (fig. 2 *a*, Pl. 14) the vitelline membrane of the posterior lobes is abundantly supplied with muscle-fibres, which have also formed in the ventral part of the anterior lobe. The fibres of the posterior lobes have already begun to diminish in number when those opposite the liver are just beginning to make their appearance. In an older larva, similar to that represented in fig. 2 *b*, Pl. 14, the fibres have almost completely disappeared from the posterior lobes of the yolk-sac, and have not yet reached their maximum development in the vitelline membrane opposite the liver. In later stages the vitelline membrane opposite the liver is quite strongly muscular, and fibres still occur in the cytoplasm of the anterior lobe when no yolk remains therein. The layer of fibres is absent altogether from those parts of the vitelline membrane which are in contact with the strongly muscular dorsal body-wall, or, in front, with the relatively rigid head-mass.

Although the occurrence of contractions of the internal yolk-sac and liver during the later stages has been observed by us, no detailed observations have yet been made on the living larva.

The other changes in the vitelline membrane at this period are closely connected with one another. One is a rapid increase in the number of nuclei per unit area of vitelline membrane; this may partly be due to an actual increase in the number of nuclei, but is certainly mainly caused by the decrease in the surface area of the yolk-sac, as the yolk is withdrawn from it, and the liver closes upon it. In the end the nuclei become so numerous that the last remnant of the yolk-sac is enclosed in a sheet of nuclei packed closely together; in this final phase they attain only about half their maximum diameter and show signs of degeneration.

The second change, synchronizing with this increase in nuclei per unit area, is a corresponding increase in the activity of the vitelline membrane and of the amount of transformed yolk present within the yolk-sac. From all the many different appearances presented in this period by the vitelline membrane, one fact, already suggested when considering Period III, becomes clear. In the vitelline membrane three conditions may be distinguished, two active, one relatively inactive. The relatively inactive state prevails in the anterior part of the membrane, where the yolk-sac is pressed against the statocysts and posterior salivary gland. Of the two active states, one is confined to the area opposite to and in contact with the liver, the other to the posterior part of the yolk-sac, which is still bathed by the posterior sinus (fig. 2 *b*, Pl. 14). As the nuclei become more and more crowded together the activity of the vitelline membrane becomes such that, in the final stages described above, no true yolk remains within the yolk-sac (fig. 2 *c*, Pl. 14).

Secretory and Absorptive Functions of the Liver.

The great growth of the liver during Period IV has already been described. A rapid increase in activity occurs simultaneously with this increase in size: the cells become greatly elongated, with expanded distal ends in which granules and droplets of various kinds appear (fig. 9, Pl. 17), while cells in mitotic division are of frequent occurrence during the period immediately following hatching. Both sides of each liver appear equally active, but that part of the liver which is in contact with the active vitelline membrane is quite clearly different from the rest. No one type of secretory cell is peculiar to either side of the liver, though some predominate on one side, some on the other; but in the part of the liver in contact with the yolk-sac are many cells stained deeply with iron haematoxylin, while on the other side such cells are infrequent. Further, that part of the liver in contact with the vitelline membrane is thicker than the rest (fig. 2 *b*, Pl. 14), and the cells are more closely packed together. In the last stages, when the yolk-sac has almost vanished and no true yolk remains, these differences between the two sides of the liver disappear.

Thus, during Period IV, the liver is performing two separate functions. One is common to the whole organ and probably represents the activity of the adult liver, the other is confined to the cells in contact with the yolk-sac. That this second, superimposed activity is, in fact, the transference of material from the yolk-sac to the interior of the animal can hardly be doubted after a consideration of the facts described in this paper. The nature of the transformed yolk when it enters the liver, and of the products delivered by the liver to the other organs of the body, is unknown.

The 'pancreas' as a Yolk-absorbing Organ.

It was stated above (p. 302) that during the early stages of development the transformed yolk passes directly into the blood-stream and thence to the organs of the body, and this process continues to the end in the posterior lobes; it would seem, therefore, as if the liver need only serve as a mechanical intermediary between the yolk-sac and the blood-system. That the vitelline membrane opposite the liver is different from that in the posterior lobes where the yolk is also being transformed and absorbed would suggest that the liver has, nevertheless, an effect on the yolk before it enters that organ: such an interaction between liver and vitelline membrane is not surprising in view of the intimate contact between the two organs.

The question which now presents itself is whether or not the transformed yolk passes directly from the liver to the blood-stream: our observations suggest a negative answer. The blood-supply of the liver has the aspect of such a purely nutritional supply as is typical for any functional organ, and is not sufficiently developed to be capable of dealing adequately with the great volume of yolk which must pass from the yolk-sac to the growing animal. We must therefore assume that the transformed yolk is mixed with the digestive secretion of the liver when the latter leaves that organ. Thus the transformed yolk must pass to the blood-stream through either the 'pancreas', caecum, stomach, or intestine. It does not appear probable that the main absorption of the yolk occurs in either caecum, stomach, or intestine, since a large quantity of yolk has already been absorbed before these organs assume a functional aspect. On the other hand, the so-called 'pancreas' is especially remarkable for its strongly developed blood-supply, which appears at a relatively early age (about the middle of Period III) as a sinus, completely surrounding and bathing the organ, and communicating with one of the posterior branches of the vena cava, the so-called afferent branchial vessel (fig. 15 *a*, *b*, Pl. 19). The sinus is still present and unmodified in the latest stage examined by us; a larva in which the yolk was completely absorbed, and which had been captured in the tow-net and thus represents normal conditions. This rich blood-supply and the large surface which is also characteristic of the 'pancreas' fulfil two conditions essential for absorption. Further, the cells of this organ differ strikingly from those of the liver. For the nuclei of the liver lie typically in the base of the cell, near the vitelline membrane, while the free or distal end is filled with the products of its secretory activity; but the nuclei of the pancreas lie typically in the distal end of the cell, which contains no traces of secretion and resembles the cells of the kidney, in its general aspect, rather than those of the liver. Finally, any fluid leaving the liver on its way to the alimentary canal must first pass through this 'pancreas', by which the absorption of the transformed yolk may thus be effected.

The function of the adult 'pancreas' in Loligo is at present entirely unknown.[1] The cells of the pancreas are all of one type and have always been looked upon as secretory. A fluid has been obtained from the 'pancreas' and examined as 'pancreatic secretion'. As it has been shown, however, to be identical in its effect with that obtained from the liver, it may be, in fact, the liver secretion, passing through the 'pancreas' on its way to the caecum.

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[1] In all that regards the functions of the adult liver and pancreas we follow the summary given by Jordan (1913).

If the 'pancreas' have no secretory activity, might it not have that function of absorption, long sought for in the liver, which has been, and is, the subject of so much controversy? There is strong evidence that the fats are absorbed in the caecum, the proteins in the intestine, and that the stomach has no absorptive function. It is not known where the carbohydrates are absorbed, and a large number of experiments have failed to detect any glycogen in the liver. Our observations strongly suggest an absorptive function in the developing 'pancreas', and may thus throw some light on the function of this enigmatic organ in the adult Cephalopod.

GENERAL CONCLUSIONS.

It is a truism that the Cephalopod development differs from that of all other groups of Mollusca on account of the large mass of yolk present in the egg, and in this respect the Cephalopoda have often been compared with the Vertebrata.

In both groups this mass of yolk alters the primitive type of segmentation from a total to a discoidal cleavage; in both groups a large yolk-sac is formed outside the embryo with a regular blood-supply which absorbs the yolk.

But in the Vertebrata, the first steps only of embryonic life are modified by this mass of yolk, while in the later stages the yolk-sac is entirely outside the embryo, with which it is connected only by the umbilical cord. Thus the yolk-sac is only an external food-reservoir, and has no direct effect on the shaping and differentiation of any organ.

In the Cephalopoda, on the other hand, owing to the presence and growth of a large internal yolk-sac, the shaping of the embryo and the differentiation of its organs are strongly modified by the yolk so long as any yolk is present. In this group, through an early and rapid development of the head region as compared with the rest of the body, the circulation of the external yolk-sac is cut off, as has already been described (p. 203), from the blood-system of the embryo. Thus the important mass of the outer yolk would no longer be available for the embryo were it not for the contractile mechanism (Portmann, 1925) which drives this yolk into the embryo, thus effecting the great development of the inner yolk-sac.

This well-known growth of the inner yolk-sac was formerly regarded merely as an interesting detail of secondary importance, since the development had never been followed beyond that point at which the organs of the body were recognizable. Our observations of the later stages show that this growth is no secondary detail, but has on the contrary a very important influence on the growth and differentiation of what we have termed the ' visceral mass '. The cephalic organs show a steady growth and differentiation throughout development. That part, however, of the alimentary canal which lies within the visceral mass (i. e. the stomach and caecum) is arrested in its development as soon as the yolk-sac begins to exert pressure upon it; and its cells remain in an undifferentiated embryonic condition, at a stage at which, for instance, the eye, brain, and buccal mass have all reached a high degree of specialization. The effect of the growth of the yolk-sac upon the form and growth of the liver has already been sufficiently stressed and needs no further emphasis.

As soon as this pressure is removed by the decrease in size of the internal yolk-sac, the development of stomach and caecum becomes very rapid, and their cells quickly show the characteristic differentiation of the adult organs. The caecum, in particular, grows to a very large size and occupies that space formerly filled by the posterior lobes of the internal yolk-sac.

Thus the internal yolk-sac has an exceedingly important morphogenetic effect upon some organs of the developing Cephalopod. But this is not all: of equal importance is the influence exerted upon the functions of these organs. For this great mass of yolk present within the developing animal has somehow to be absorbed. And thus the liver and pancreas, while developing into active adult organs, have, in addition, to perform for a time the function of yolk absorption.

As with the Cephalopoda among the Mollusca, so, in many other groups of animals, a primitive development is modified by the presence of large quantities of yolk. In no other group, however, has it such a profound effect upon the growing animal. The problem of how to absorb the yolk has been met in many very different ways: the Cephalopoda are unique in that, with them, two separate special mechanisms perform successively the absorption of the yolk.

SUMMARY.

1. The development of Loligo (as of Cephalopoda in general) is characterized by the large quantity of yolk present within the animal until a post-embryonic stage when development is far advanced.
2. Two separate mechanisms are successively employed for the absorption of the yolk: in one the blood-system only is involved, in the other the nutritive material passes from the yolk-sac into the liver.
3. The liver, throughout almost the whole of its development, is directly and closely in contact with the yolk-sac, which it finally replaces in the body of the animal. The so-called 'pancreas' is in direct communication with the liver, and is surrounded by a blood-sinus. Evidence is given that the nutritive material passes from the liver to the blood-stream through the 'pancreas'.
4. The absorptive function claimed for the embryonic liver and 'pancreas' is of especial interest in view of the controversy which exists concerning the possibility of absorptive function in the adult organs.
5. A comparison of the development of Loligo with that of other yolk-laden eggs reveals the unique complexity of the yolk-absorption in this animal, a complexity which is probably typical of the Cephalopoda.

[The special thanks of one of us are due to Professor Zschokke for permission to work in his laboratory at the University of Basle, and for his constant kindness. A. M. B.]

APPENDIX A.

Rearing Experiments.

The difficulties of keeping the post-embryonic stages of Loligo alive in captivity are well known. One of us succeeded at Plymouth in keeping the larvae alive for a week, until the yolk was completely absorbed. The larvae then all died, almost certainly of starvation. As we believe that most workers have not succeeded in keeping the larvae alive even for this length of time, we append here the results of our experiments, in the hope that they may prove of service to any who are faced with the same problem.

In the course of experiment it appeared essential that:

(1) The water in which the larvae are kept should be exceedingly clear, and as free as possible from any solid matter. The bottom of the containing vessel should also be as clean as possible, as larvae often sink for a while to the bottom and swim slowly along it, during which process their mantles rapidly become wounded and infected by any detritus present.

(2) A 'healthy' culture of diatoms on the walls of the containing vessel is not beneficial to the larvae.

(3) The containing vessel must be kept cool, standing, if possible, in running water. It should not be in too bright a light.

(4) A 'plunger' or circulation of air alone seemed to be equally effective, though the plunger may have given slightly better results. If an air circulation is used the current must be so gentle that the surface of the water is not perceptibly disturbed.

(5) The larvae must, of course, be few in number: the largest number taken was 70 larvae in a bell-jar holding about 25,000 c.cm. It is possible that this number might be increased without detriment to the health of the larvae. Egg-capsules must not be kept in the same vessel as hatched larvae.

(6) The following food materials were tried without success:

- (i) Small Crustacea from 'medium' tow-nettings, chiefly small copepods.
- (ii) Diatoms.

(iii) Fish larvae.
(iv) Oyster veligers.
(v) Phaeocystis.
(vi) Pomatoceros trochospheres.

It may be stated definitely that diatoms by themselves were unsuccessful. With all the other food material it is probable that the cultures used were not sufficiently dense to ensure the larvae finding the food. One of the problems to be met is how to combine sufficiently rich cultures of food with the necessary clearness of water.

Hardly any direct evidence of larvae-feeding has been obtained. One larva was observed to take up oyster veligers and drop them, as if in disgust, and one was taken from a bell-jar holding a copepod (Temora longicornis), which it appeared to be eating, in its arms.

APPENDIX B.

Prenant's Method as used by us differs from that given by Langeron. We owe our modification to Professor O. Duboscq, Director of the 'Laboratoire Arago' at Banyuls-sur-Mer; and we describe it below as we found it gave excellent results, not only with Cephalopod embryos and larvae, but with very varying kinds of material.

Fixatives containing formol proved unsuitable for use with this method.

(1) Stain with iron haematoxylin by Heidenhain's method.

(2) Wash as usual in tap-water, not less than $\frac{3}{4}$ hours.

(3) Wash in distilled water.

(4) Stain in solution of eosin in distilled water (1 per cent. solution diluted to deep salmon pink) for 24 hours.

(5) Rinse with distilled water.

(6) Fix eosin with 1 per cent. solution of phosphor-molybdic acid 5–10 minutes.

(7) Rinse in tap-water.

(8) Stain in 0·25 per cent. aqueous solution of Lichtgrün 2–3 minutes.

(9) Rinse quickly through 65 per cent. and 85 per cent. alcohol.

(10) Differentiate in 95 per cent. alcohol, on microscope stage, until the preparation shows bright red-and-green staining.

(11) Mount in Canada balsam.

LIST OF AUTHORS TO WHOM REFERENCE IS GIVEN IN THE TEXT.

Bobretzky, N.—" Untersuchungen über die Entwicklung der Cephalopoden ", 'Nachr. d. Ges. d. Freunde d. Naturw.', **24**, 1877. (Moscow, Russian.)

Faussek, V.—" Untersuchungen über die Entwicklung der Cephalopoden ", 'Mitt. d. Zool. Stat. zu Neapel', **14**, 1901.

Jordan, H.—'Vergleichende Physiologie der wirbellosen Tiere.' Jena, 1913.

Korschelt, E., und Heider, K.—'Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Tiere.' Jena, 1893.

Langeron, M.—'Précis de microscopie.' Paris, 1925.

Lankester, E. Ray.—" Observations on the Development of the Cephalopoda ", 'Quart. Journ. Micr. Sci.', New Ser., **15**, 1875.

Naef, A.—" Die Cephalopoden ", 'Fauna u. Flora d. Golfs v. Neapel', **35**, I. Teil, 1921, 1923.

Portmann, A.—" Der embryonale Blutkreislauf und die Dotterresorption bei Loligo vulgaris ", 'Zeitschr. f. Morph. u. Oekol. d. Tiere', **5**, 1926.

EXPLANATION OF PLATES 13–19.

REFERENCE LETTERS.

A, aorta ; *AL*, anterior lobe of the internal yolk-sac ; *AP*, anal papilla ; *B*, blood ; *BM*, buccal mass ; *Br*, brain ; *BV*, afferent branchial vessel (posterior limb of vena cava) ; *C*, caecum ; *CM*, mesoderm cells ; *E*, eye ; *EY*, external yolk-sac ; *F*, funnel ; *FR*, retractor muscle of funnel ; *G*, gill ; *GH*, gill-heart ; *I*, ink-gland ; *K*, kidney ; *L*, liver ; *LC*, LC^1, liver cells ; *LS*, secretion of liver ; *M*, mantle ; *MC*, mantle cavity ; *MF*, muscle-fibres ; *MG*, midgut ; *Mo*, mouth ; *NY*, remains of neck which joined internal and external yolk-sacs ; *Oe*, oesophagus ; *P*, pancreas ; *PL*, posterior lobe of the internal lobe of the internal yolk-sac ; *PS*, pancreatic sinus ; *S*, stomach ; *SG*, posterior salivary gland ; *Sh*, shell-gland ; *SP*, posterior sinus ; *St*, statocyst ; *SY*, sinus of external yolk-sac ; *TY*, transformed yolk ; *V*, ventricle ; *VC*, vena cava ; *VM*, vitelline membrane ; *X*, point where oesophagus and aorta pass between the posterior lobes of the internal yolk-sac; *Y*, yolk; *YN*, yolk-nucleus; *YP*, yolk-plate; *Z*, layer of liquid between liver and vitelline membrane.

In the following descriptions the time elapsed between the hatching of a larva and its fixation is given when known, but these figures give no accurate measure of the stage of development owing to great individual variation in the rate of yolk-absorption.

PLATE 13.

Fig. 1.—Embryo and larva of Loligo drawn as transparent objects to show the yolk-sac and liver: (*a*) and (*b*) represent an embryo at Stage XVI of Naef (beginning of Period III); (*b*) and (*c*) represent a larva at the end of Period III. (*a*) and (*c*) ventral, (*b*) and (*d*) lateral view. The anal papilla, gills, and visceral mass, indicated in (*b*) and (*d*), have been omitted from (*a*) and (*c*) in order to show the yolk-sac more clearly. The lines A and B show the planes of section of Fig. 5 (*a*) and (*b*).

PLATE 14.

Fig. 2.—Horizontal sections through three larvae of Loligo hatched (*a*) 4–10 hours, (*b*) 54–73 hours, (*c*) more than 120 hours. The section was in each case selected to show as much as possible of the yolk-sac. In (*b*) transformed yolk is visible underlying the vitelline membrane; in (*c*) no true yolk remains. The figure also shows growth of the liver, thickening of that organ next the yolk-sac (*b*), and formation of liver tubules (*a*) and (*c*). The thin walls of the tubules in (*c*) probably show a starving or unhealthy condition, as, in a healthy larva, taken in the tow-nets, of a slightly later stage, the liver-cells are so elongated, and their distal ends so expanded, that they fill almost the whole lumen of the organ.

PLATE 15.

Fig. 3.—Diagrammatic sagittal section through an embryo of Loligo during Period II to show the general anatomy and the circulatory system of the yolk-sac at its maximum development. Several organs not situated in the sagittal plane are shown. The figure shows the absence of any direct connexion between yolk-sac and alimentary canal.

Alimentary system in black; yolk, dark grey; blood, pale grey.

Fig. 4.—Diagrammatic representation of the organs in the mantle cavity of a larva of Loligo vulgaris at the time of hatching.

Alimentary system in black; circulatory system, white.

PLATE 16.

Fig. 5.—Transverse sections through the embryo of Fig. 1 as indicated by lines A and B; (*a*) through the anterior, (*b*) through the posterior lobes of the internal yolk-sac.

Fig. 6.—Transverse section through a larva taken in the tow-nets, and therefore presumably normal and healthy. This figure, together with Fig. 5 (*a*), shows how the liver replaces the yolk-sac.

PLATE 17.

The detailed drawings were all made with a Zeiss 1/12 oil immersion and Zeiss ocular No. 4 (X × 10).

Fig. 7.—Liver-cells of an embryo of Loligo vulgaris of about Stage XVII of Naef. The vitelline membrane is thin, the nuclei of the liver-cells are large, a layer of mesoderm-cells and blood intervenes between liver and yolk-sac, and secretion in the liver has begun.

(Fixed, Bouin; stained, borax carmine, Bismarck brown, bleu de Lyon.)

Fig. 8.—Liver-cells and part of the yolk-sac of the embryo shown in fig. 14. The nuclei have become smaller, more secretion is present in the lumen of the liver, and the intervening mesodermal layer is reduced to scattered cells. The figure also shows one of the smaller vessels of the hepatic blood-supply (B). This was, in its staining, clearly different from the layer *Z* which underlies the liver, confirming our view that this layer is part of the vitelline membrane, and represents the products of its activities. The large yolk-nucleus presents the size and appearance characteristic of the active vitelline membrane.

(Fixed, Zenker; stained, haemalum, orange G.)

Fig. 9.—Liver-cells, showing different stages of activity, from a horizontal section of a larva of Loligo media hatched 73–96 hours. In some cells (*LC*) the middle portion is laden with iron haematoxylin, while green or pink droplets appear in the distal end; in others (*LC′*) the distal ends alone stain deeply with haematoxylin, and no droplets are visible. The yolk-nuclei are much reduced as compared with those of figs. 7 and 8. A comparisòn with fig. 12 shows the specialized condition of the vitelline membrane opposite the liver.

(Fixed, Zenker; stained, iron haematoxylin, and counterstained with Prenant's method; see Appendix B.)

Fig. 10.—Liver-cells of the free-swimming larva (Loligo media) from which figs. 6 and 15 *b* were taken, showing great activity, heavy staining with iron haematoxylin, and many ' vacuoles ' or droplets staining green in every cell.

(Fixed, 5 per cent. formol; stained, iron haematoxylin; counterstained, lichtgrün in alcohol.)

PLATE 18.

Fig. 11.—Transverse sections of the vitelline membrane of an embryo of Loligo vulgaris late in Period III; (*a*) opposite the liver (indicated in outline), (*b*) opposite the body-wall. The vitelline membrane opposite the liver is different, both in width and nature, from the rest.

(Fixed, Flemming without acetic; stained, iron haematoxylin.)

Fig. 12.—Section through the vitelline membrane of a larva of Loligo media hatched 4–10 hours; (*a*) through the junction of anterior and posterior lobes, (*b*) through one of the posterior lobes. The strongly developed musculature is seen in longitudinal section in (*a*), cut transversely in (*b*). In (*b*) some yolk-plates are present in which the centre is stained with iron haematoxylin. In preparations stained by Prenant's method these plates always have a pinkish tinge, while the rest of the yolk is bright green.

(Fixed, Zenker; stained, iron haematoxylin; counterstained, Prenant's method; see Appendix B.)

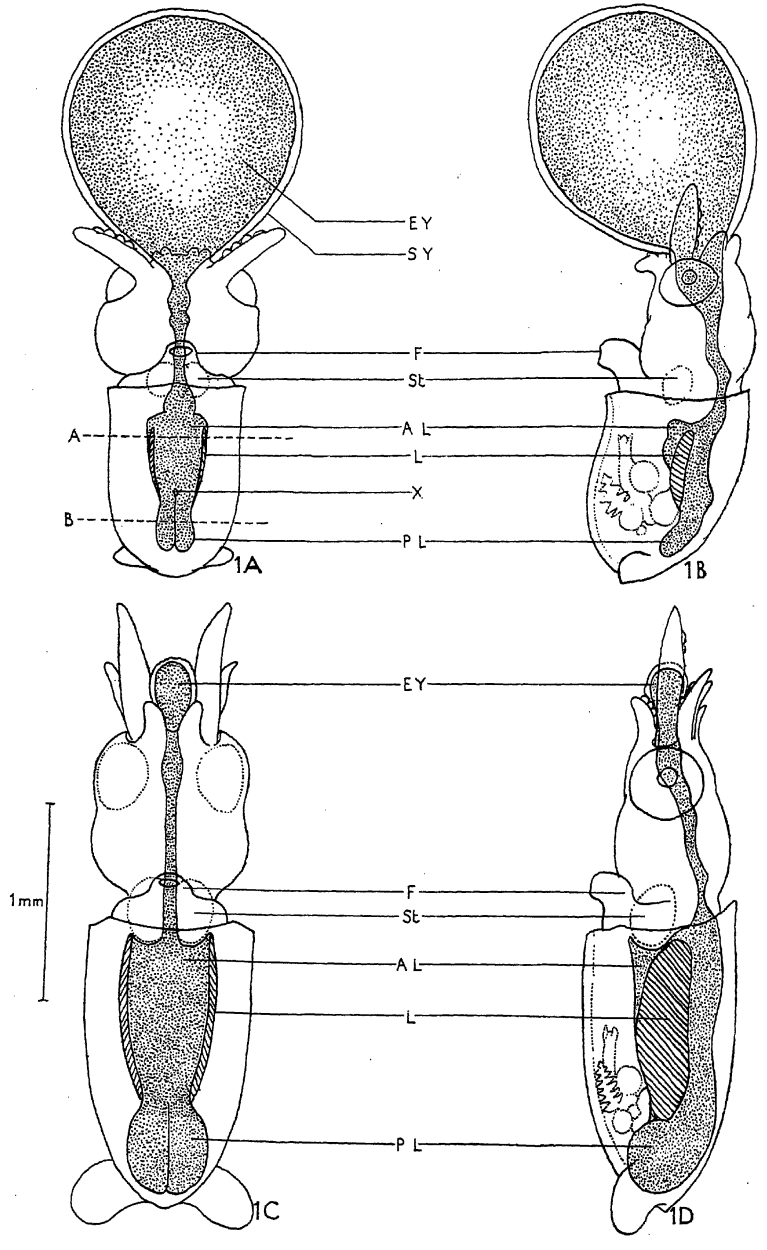
PLATE 19.

Fig. 13.—Transverse section of an embryo of Loligo vulgaris (Stage XIII of Naef) showing part of the yolk-sac and one lobe of the liver. The liver is completely surrounded by mesoderm cells and bathed in the sinus surrounding the inner yolk-sac. Its form is still almost unaffected by the presence of the inner yolk-sac.

Fig. 14.—Transverse section through an embryo of Loligo vulgaris late in Period III. The liver is much compressed by the yolk-sac. The layer of mesoderm cells is still present but broken. Secretion is present within the lumen of the liver.

Fig. 15.—Parts of transverse sections through two larvae of Loligo media. (*a*) From a larva hatched 54–73 hours, (*b*) from the more advanced larva of fig. 6. Opening of liver into pancreas; pancreas surrounded by pancreatic sinus; opening of the latter into afferent branchial vein.

The section is designed to show the essential difference between the cells of liver and pancreas in both stages.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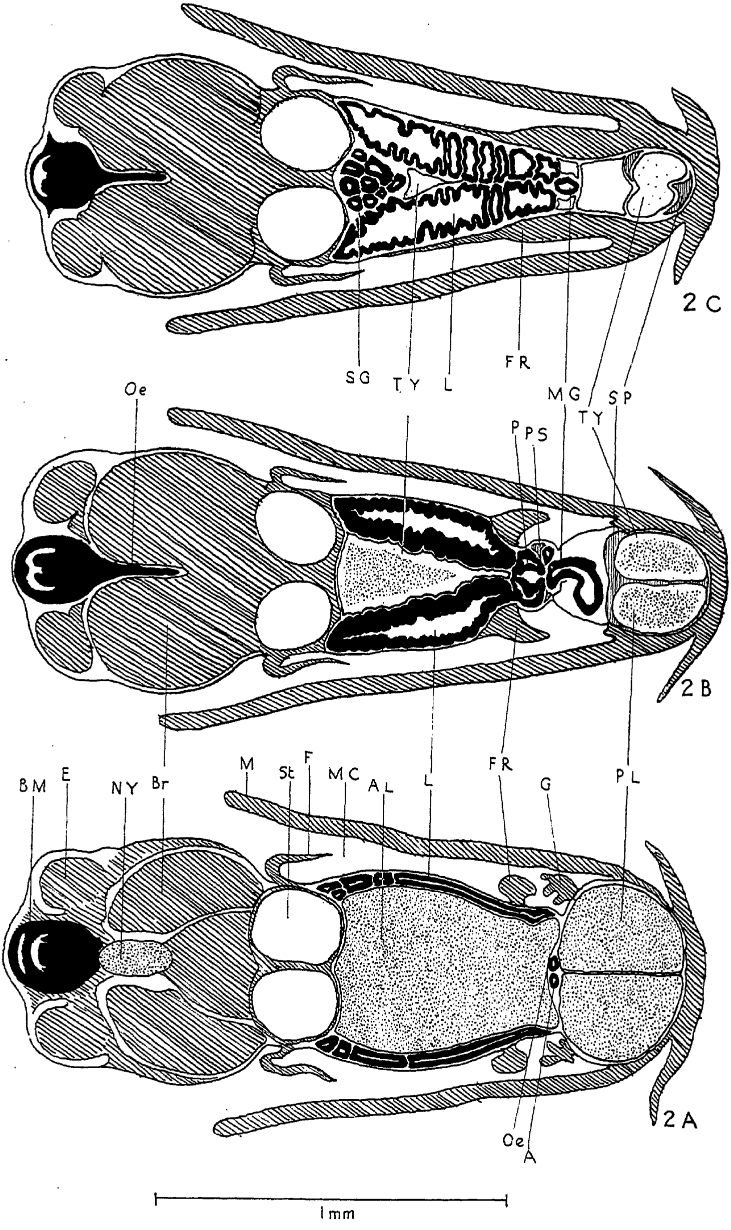
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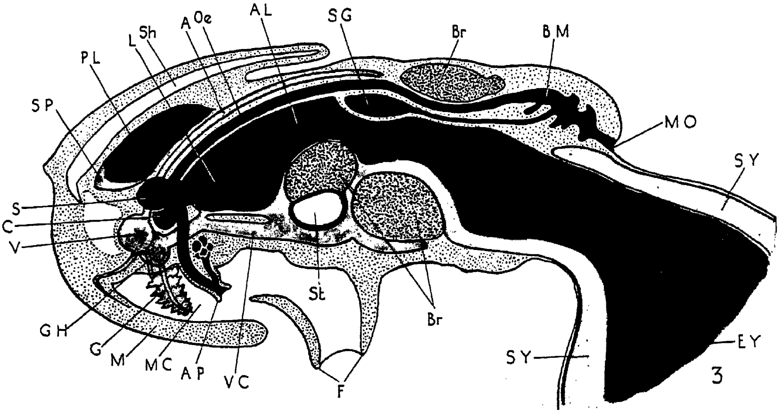

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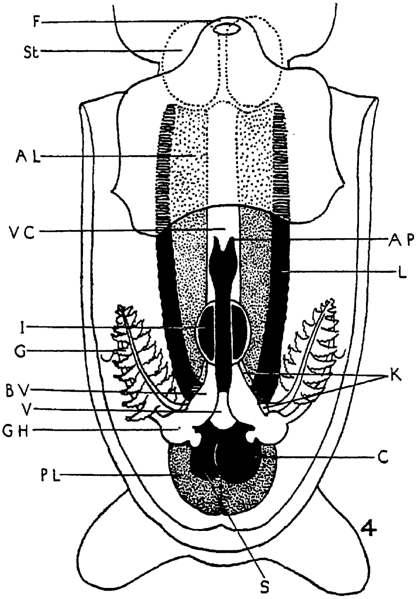

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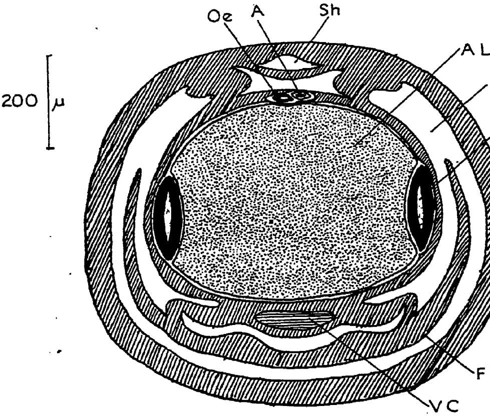
Portmann and Bidder.



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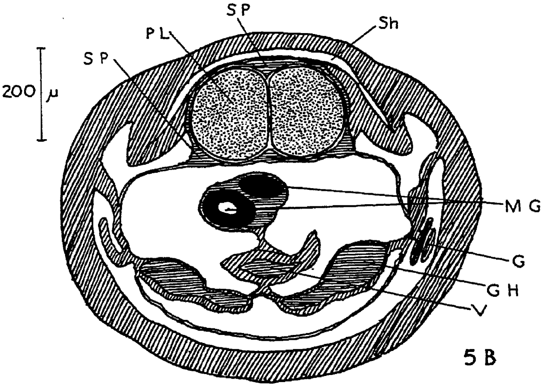
5 A



S P
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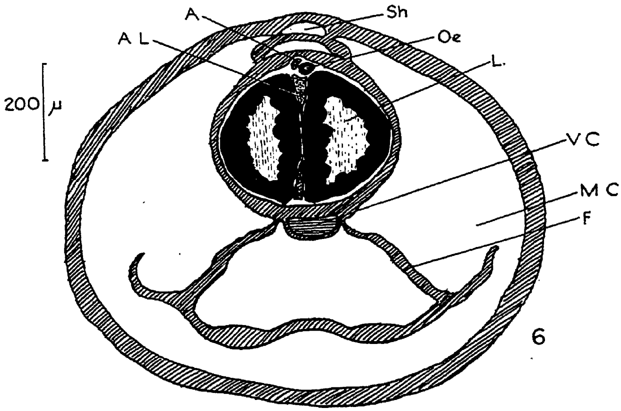
G H
V
200 μ

5 B



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V C
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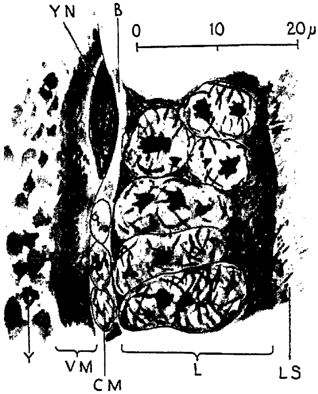
6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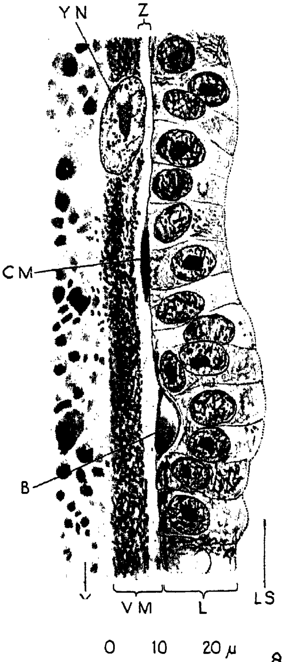
7


YN
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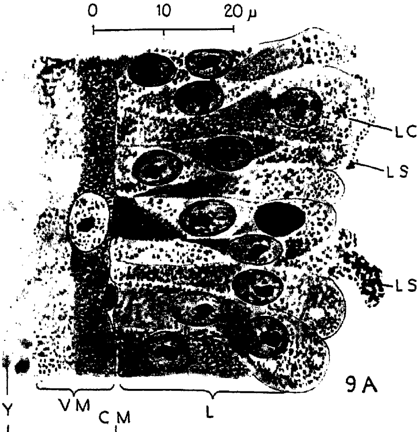
10
20 μ

8


0
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Y
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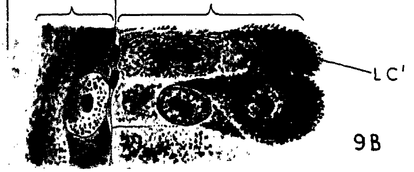
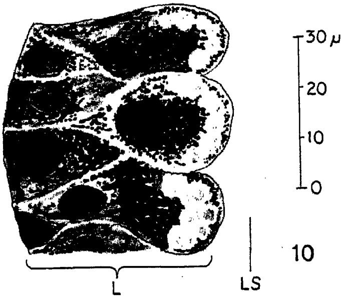
CM
L

9 A


LC'

9 B


30 μ
20
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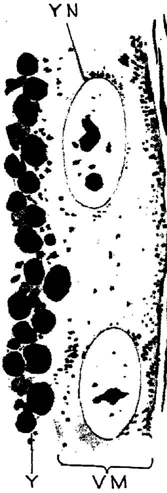
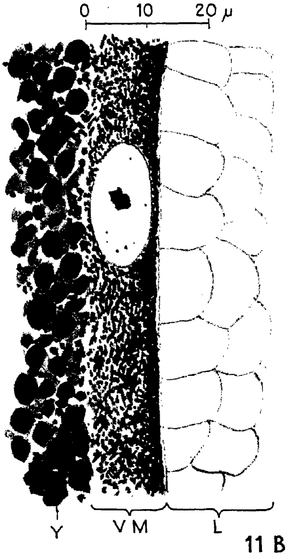
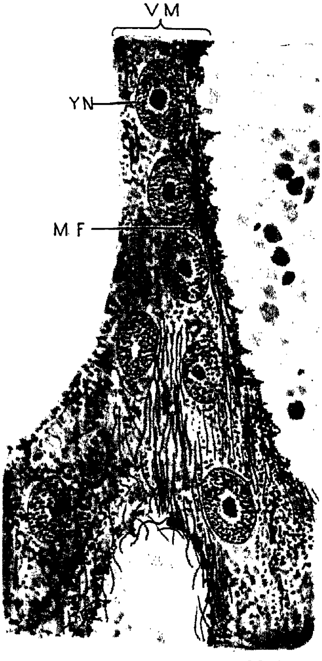
10



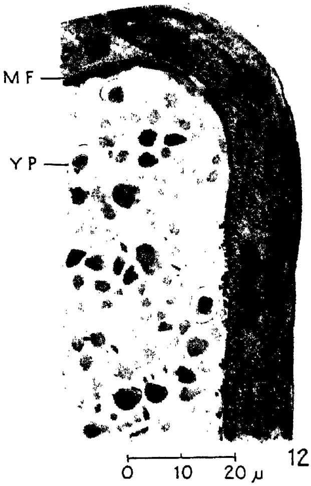
11 A



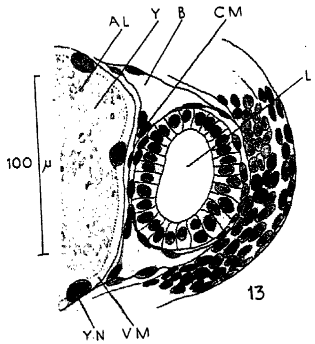
11 B



12 A



12 B


AL
Y
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100 μ
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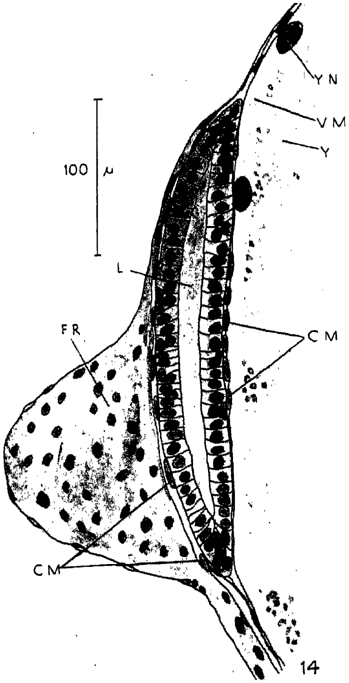
13


YN
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100 μ
L
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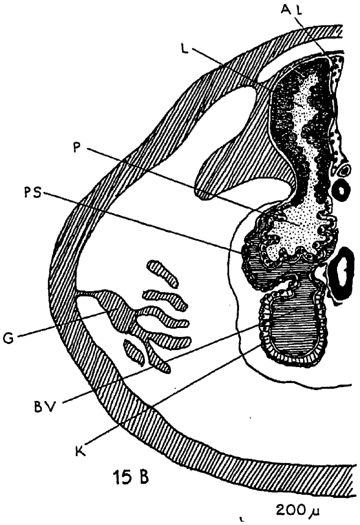
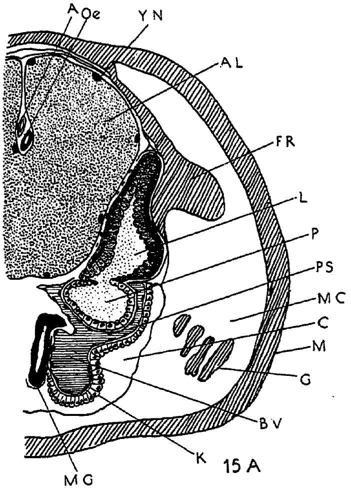
CM

14


AL
L
P
PS
G
BV
K
200 μ

15 B


A
Oe
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BV
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MG

15 A

Reactions of Gammarus to Injury and Disease, with notes on some Microsporidial and Fungoid Diseases.

By

Helen Pixell Goodrich, M.A. (Oxon.), D.Sc. (Lond.).

With Plate 20.

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SINCE May 1923 numerous specimens of the fresh-water amphipod Gammarus pulex L., from various parts of England, have been kept in my laboratory, and I have made observations on some of the many diseases to which this species is subject. The investigation was started owing to an epidemic of microsporidiosis occurring in Gammarus brought to the Department from Liverpool by Mr. C. S. Elton.[1] He and others were using these animals for ecological and genetical experiments, and the disastrous loss of offspring from disease was stopped when the nature of the epidemic was recognized. The infecting organism was Thelohania mulleri, the life-history of which is to a great extent known (Stempell, 1902; Léger and Hesse, 1917). There are certain points, however, that still require careful investigation, especially with regard to the propagation of the disease. The following incidents, which came under my observation, throw a little light on this problem. In October 1924 there was a second small outbreak of this same microsporidial disease among some Gammarus chevreuxi, Sexton, which had been bred in the department for three or four generations at least. I am indebted to Mr. E. B. Ford for drawing my attention to this outbreak among his specimens and for handing over to me any that succumbed to it. In two of these the disease had reached the spore stage, and could therefore be definitely recognized as due to Thelohania: one of these specimens had been hatched 21 weeks, the other only 16 weeks. The Gammarus chevreuxi were attaining sexual maturity at that season in from 6 to 7 weeks.

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[1] All the Gammarus from Liverpool mentioned in this paper, as well as some from Port Meadow, were obtained by Mr. Elton, and I am grateful to him also for giving me much useful information about the habits of Gammarus pulex and the possibilities of keeping them in normal

It is difficult to account for this second outbreak unless the spores from the first survived for a considerable time—at least 6 months and more likely 13 months or more [1] in the department—just possibly in the small glass bowls in which we keep

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health in laboratory tap-water. I am much indebted to Lady Muriel Percy for obtaining many Gammarus for me from Albury, Guy's Cliffe, Patshull, Hodnet, as well as many places in this neighbourhood. Professor E. S. Goodrich, in whose Department the work has been done, has also given me much valuable assistance.

[1] All my specimens from Liverpool were dead by September 1923, and I understand from Mr. Elton that he had no more Gammarus pulex from this infected area till after this outbreak. He, however, kept some Gammarus duebeni Lilj. (another brackish-water species) in his laboratory until about April 1924, when all Liverpool material was thrown away. No case of microsporidiosis was noted during this time, so it does not seem likely that Mr. Ford's specimens in another laboratory could have been infected from Liverpool at all. Nor have we had any infected specimens from Plymouth. In fact until 1926 I had had no other Gammarus infected with Thelohania. It is only since then that three specimens containing spores of this parasite have been found locally, and that Gammarus from Albury and Patshull have also been found to suffer occasionally from this disease.

our Gammarus, these being washed only after use, not sterilized.

Another fact noticed is that although infection is supposed to be contaminative, spores have been found to pass through the alimentary canal unopened and apparently unchanged. As occasion arises, I am making further observations on this and another microsporidian disease met with in local Gammarus due to Nosema, not, so far as I can discover, recorded before. Both of these microsporidia have merozoite stages free in the haemocoelic fluid where rapid schizogony takes place. At the end of this multiplicative stage the sporonts find their way into the muscles between the contractile fibres. In Nosema only a single spore is formed from each sporont,[1] and these spores may be seen lying in rows between the fibrils of an infected muscle-fibre (fig. 1, Pl. 20) causing apparently very little inconvenience to the host. It is only in exceptional circumstances (mentioned below, p. 339, under phagocytosis) that the host takes any steps for their removal.

There are numerous other parasites more or less pathogenic to Gammarus that are frequently met with, but I wish to deal chiefly in this paper with a yeast-like organism which is liable to infect the blood and prove fatal.

YEAST DISEASE.

The yeast-like organisms which, for the present, will be referred to simply as yeasts, are oval while actively budding (figs. 2 *a*, 3 *a*, *b*, Pl. 20), but later become rod-like (2 *b*, 3 *c*) or sometimes slightly curved. The buds soon separate, so that no strings of parasites are found. While living they are all refringent, and little structure can be made out except the vacuoles. Neutral red as an intra-vitam stain has a striking reaction, being absorbed very readily by the numerous vacuoles which become brick red (alkaline reaction) while the rest of the organism remains colourless (fig. 2 *a*, *b*, Pl. 20). Further, near each end of the rod-like forms is a rounded body which remains refringent and colourless and sometimes shows a rosette appearance (fig. 2 *b*, Pl. 20).

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[1] The spore stage in the Gammarus muscle is therefore quite distinct from that of Thelohania, where eight spores are developed from a single sporont and remained held together by the parent membrane (pansporoblast stage.)

In size the yeasts vary much: the oval form may attain a length of 10μ, the rod-like ones are generally $8–12\mu$ long.

The yeasts may swarm to such an extent in the blood that it appears almost solid with them and gives the Gammarus an opaque whitish appearance. The extremities of appendages sometimes become quite clogged with the parasites, or at most a tiny trickle of blood can flow down one side and up the other.

During parts of each year 1923 to 1928 inclusive the disease has occurred in specimens of Gammarus pulex in a stream in Oxford, a small branch of the Thames, running along Port Meadow between it and the G.W.R. main line near Walton Well Bridge.

Something approaching to an epidemic has generally appeared in the early autumn (when, no doubt, the Gammarus population is at its maximum) and continued until May or June, when it has died down or disappeared temporarily. Infected specimens were especially looked for in June 1925 and again in 1926 during the months May to August, but none were found. The epidemic was specially severe in the autumn of 1925, when 54 per cent.[1] of the specimens examined in September were infected with this disease alone. By the end of January 1926 only 18 per cent. were infected, and on May 12 that year no diseased specimens could be found at all, nor in June, July, or early August.

Unfortunately in the latter part of August 1926 this stream was cleared out by dredging, and practically all weed and incidentally all Gammarus removed. The pH was found on September 3 to have fallen from 7·6, its usual value, to below 7, and no specimens at all could be found—either healthy or diseased—during the rest of that year.

However, Gammarus reappeared in this stream early in

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[1] These percentages have only a rough comparative value, for of course all the specimens were more or less selected.

1927, and some were obtained in March, when the floods were out and the pH was approximately 7·8. Specimens from the stream were collected for examination about once a month. The first reappearance of yeast disease was in July in a specimen which had been in my laboratory for a month. From October 1927 to May 1928 infected specimens have been brought in at intervals, and there are generally a few to be found in the stock vessels—shallow enamelled iron trays in which the Gammarus will live in the laboratory for months without attention.

In addition to this Oxford centre of yeast disease, a few specimens from Hodnet, Shropshire, in June 1925 had the same disease. I also found two Gammarus duebeni from Liverpool with apparently the same yeast in March 1925, and, if my identification of the parasite is correct (see p. 344), it has also been common in Switzerland.

CYTOLOGICAL METHODS.

For examination of the living tissues and parasites of Gammarus its blood is the best fluid, but failing that 0·75 per cent. sodium chloride is satisfactory as an isotonic solution.

Nearly all the usual fixatives and stains used in protozoology (such as those given in the 1928 edition of the 'Microtomist's Vade Mecum') have been employed at one time or another.

Except with recently moulted specimens there is always difficulty in obtaining perfect sections of the host, though sometimes double embedding is successful. On the whole, I prefer to use warm Schandinn's fluid or Bouin's alcoholic mixture. In the latter it is easy to dissect out the abdominal organs (that is the alimentary canal with its glands together with the gonads, all more or less held together by the branches of the phagocytic organ and fat body) and of these to obtain really good sections.

The most useful method of staining for showing up the yeasts in sections is certainly Claudius's modification of Gram's stain, after colouring the tissues with carmine. The organisms are then conspicuously stained bright blue.

The yeasts themselves do not stain with carmine ; with iron haematoxylin one granule, situated generally on the edge of a vacuole, takes the stain more deeply than the others and is presumably the nucleus—sometimes there are two or more of these chromatic granules (fig. 3 *c*, Pl. 20).

DIAGNOSIS OF DISEASE.

When a Gammarus is infected with a blood parasite it is more or less opaque, the blood itself appearing milky owing to the reflection of light from the bodies in it. However, opacity does not necessarily mean disease, for specimens, which are apparently healthy, sometimes appear milky white, especially about the time of moulting. The blood at this time has often been found to be crowded with leucocytes only. For accurate diagnosis of the different diseases, it has been found best to examine the blood microscopically by snipping off the tip of one of the appendages with a sharp pair of scissors. The Gammarus, being accustomed to such small accidents in everyday life, generally take little notice, and will sometimes continue to feed during or directly after the operation. Special symptoms by which yeast-disease may be recognized will be mentioned below.

REACTIONS OF GAMMARUS TO AMPUTATION OF ITS APPENDAGES.

When part of a leg or other appendages of a healthy Gammarus is cut off with sharp scissors, haemorrhage is soon arrested, the wound being, as one would expect, plugged with leucocytes. Tait (1908, 1910) and Winterstein (1925) give some account of this early agglutination of the blood corpuscles.

Subsequent to this, in the course of a few hours, I find that a yellowish amorphous substance, looking like chitin, appears in this plug and gradually forms a continuous layer or partition. After some 24 hours this partition is found to be joined at its circumference to the chitin of the appendage. The junction is generally a short distance from the cut end of the chitin (figs. 5 and 6, *dc*, Pl. 20).

The yellowish chitinoid substance of the partition gradually becomes dark brown. Thus a dark cap, appearing black by transmitted light, is formed to the truncated appendage (fig. 4, Pl. 20).

This appearance is retained until after the next moult, when the black cap is generally thrown off with the rest of the chitinous covering, and the extremity is again the usual pale colour, i. e. covered by ordinary chitin, though it may not be until after several moults that the limb attains its normal size and shape. The one exception to this is when the Gammarus is cut within a day of being ready to moult: the partition is then found as usual and becomes brownish, but is joined in the course of 24 hours to the new chitinous covering of the Gammarus and therefore persists till the moult after the one that is due.

Exactly the same reaction appears to take place when a Gammarus is injured accidentally or intentionally by another animal, and consequently it is by no means uncommon to find black ends to appendages. Even gills may have black edges or extensive patches, indicating healed wounds, or there may be black scars showing entire loss of appendages or injury to other parts of the body. Pantel (1910)[1] mentions similar black scars formed in larvae of insects, and briefly refers to them as cicatrices surrounded by 'chitine pathologique' (p. 165). He does not apparently explain what he means by this, but the term—pathological chitin—would be suitable if adopted to mean that the animal's leucocytes help in its formation. With regard to the nature and origin of the dark amorphous substance which closes these wounds, and so closely resembles chitin, I shall have more to say below after some observations on phagocytosis. From the examination of sections carefully cut longitudinally through limbs fixed at intervals from 1 hour to 23 days after amputation of their extremities, it certainly seems as though this chitinoid substance were formed chiefly of flattened cells themselves containing the yellow chitinoid secretion which gradually darkens. In stained preparations the flattened nuclei (figs. 5 and 6, *n*, Pl. 20) can be made out embedded in this substance, especially clearly in sections stained with carmine. Occasionally a few phagocytes containing yellowish refringent globules can be seen on their way to the partition (fig. 5, *p*, Pl. 20), but the product only appears in quantity in cells which are already necrotic.

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[1] I am much indebted to Dr. Keilin, F.R.S., for drawing my attention to Pantel's papers on insects and for other help in connexion with this paper.

As shown in fig. 5, Pl. 20, a section through the healed end of a leg 21 hours after snipping off its extremity, the chitinoid substance has made a more or less continuous partition and is already darkening. The regenerating epidermis (*e*) is to be seen at the side growing across underneath the dark cap: some days later these epidermal cells secrete a layer of ordinary chitin which may be seen lying underneath the dark chitinoid substance—especially clearly in longitudinal sections of healed legs stained by Mallory's method.

It should be noticed that the chitin of the appendage has itself darkened just where the partition is forming (figs. 5 and 6, *dc*, Pl. 20), presumably owing to some secretion from the phagocytes. In the course of a day or so the terminal fragment of chitin generally falls away with the rest of the necrotic tissue exterior to the partition, and the cap is then smooth on the outside (fig. 4, Pl. 20) and apparently continuous with the ordinary chitin of the appendage.

When a Gammarus with yeast disease is cut, large numbers of yeasts escape, and haemorrhage is only arrested after a varying time, that is, when enough leucocytes have accumulated at the spot to form a plug. The subsequent behaviour during healing of the wound is apparently the same as in a healthy specimen. Only once or twice have I had Gammarus which did not recover, i.e. which bled to death after snipping, and these were in very advanced stages of the disease. In one of the cases the leg had been amputated at its widest part close to the body, making healing specially difficult.

Healing also takes place, as a general rule, in specimens of Gammarus whose blood is crowded with microsporidian merozoites. In these diseases also it seems likely that Gammarus only bleeds to death if injured when already moribund.

Further, Gammarus is capable of severing its own limbs under certain circumstances such as injury (or disease, as will be pointed out below). This voluntary amputation or autotomy is generally effected, however, at the joint proximal to the injury. The area to be covered by the plug and chitinoid partition is smaller at joints than elsewhere. The end distal to the partition then becomes opaque, withers, and in time falls off, leaving the stump of the appendage with its usual black cap.

PHAGOCYTOSIS IN (1) YEAST DISEASE.

The fight against the yeasts is very actively carried on by the phagocytes of Gammarus, and there is no doubt that under natural conditions they are often successful and the animal recovers—not always, however, for dead and dying specimens have sometimes been brought in from the stream. Even in the laboratory I have had one or two specimens which have recovered from an infection when kept under conditions approaching the ideal—water constantly renewed and plenty of food, weed, &c. Further, owing to the activity of the phagocytes, artificial inoculation is often unsuccessful, as will be pointed out below.

There appear to be two kinds of leucocytes in the blood which ingest the yeasts (fig. 7, *a* and *b*, Pl. 20). I have not attempted to follow the development of these blood corpuscles, which would, no doubt, be a very interesting study. Bruntz (1907, p. 17) has discovered lymphoid tissue (' l'organe globuligène ') in the anterior dorsal region of the head of Gammarus. He describes corpuscles of three shapes or sizes, but does not mention the so-called explosive leucocytes (thrombocytes) which have been described elsewhere as helping in agglutination (Winterstein, 1925) in some species of Gammarus. So far as I have studied them, all the corpuscles, when floating freely in the blood or an isotonic solution, are more or less elongated and fusiform owing to folds of the membranous pseudopodia (Goodrich, 1919) projecting from one or both ends. These folds form a kind of funnel in which the yeasts appear to be caught. Presumably it is at the base of the folds that the parasites are subsequently ingested in larger or smaller numbers. When creeping on glass the pseudopodia become lobose, as shown in fig. 7, Pl. 20.

The smaller of the two kinds of phagocyte (fig. 7 *a*) measures 16–18μ in diameter and has fairly clear cytoplasm. If refringent globules are present they are few in number and grouped round the nucleus, which is spherical and about 8μ in diameter. There is little doubt that the forms represented in fig. 8 are later stages of this phagocyte, and show that acting alone they are by no means always able to cope with their parasites. I have seen a phagocyte appearing to digest one or even two yeasts, but when more than three or four parasites have been ingested the phagocyte nearly always shows necrotic changes: the nucleus degenerates and the spaces round the ingested yeasts tend to coalesce. When ten or more parasites are present in one blood corpuscle there is usually only a membrane left, together with a distorted nucleus crushed to one side (fig. 8 *b*).

The other kind of leucocyte sometimes found containing yeasts (fig. 7 *b*, Pl. 20) is slightly larger (up to 24μ in diameter), and its cytoplasm is crowded with refringent globules, leading one to suppose that the secretory function is predominant. Now certain phagocytes deal with their parasites by embedding them in a clear chitinoid substance which gradually darkens (figs. 9 and 10, Pl. 20). It is tempting to suppose that this reaction is effected by these corpuscles already full of a secretion which possibly only requires little change. However, I have no evidence in support of this theory apart from appearance, and shall have to refer to this point in more detail below.

There is no reason to think that the yeasts multiply inside any of the blood corpuscles, as do Leishmania and some other protozoa in certain vertebrate leucocytes. I have seen a yeast with bud attached being engulfed, and it seems likely that any budding forms found inside phagocytes have been ingested in that condition.

When a host is making any headway in dealing with its parasites the phagocytes agglutinate to some extent and deal co-operatively with the yeasts. Aggregations of three or four are frequently seen, and attached to their periphery may be other leucocytes without any parasites. Small aggregations, like this, may be quite colourless and transparent; on the other hand, they often contain one or more corpuscles that have already embedded their parasites in a chitinoid secretion. We find, in fact, all stages of aggregations of phagocytes forming nodules. Somewhat similar nodules (' nodules leucocytaires ' of Cuénot, 1898) are, of course, common in many worms, echinoderms, &c. All that I have hitherto studied have, however, been soft opaque masses, generally oval and made up of numerous leucocytes together with parasites and waste products from the body cavity—especially brownish granules—giving a dark colour to the whole mass. They remain, however, quite soft and compressible, and are easily teased out with fine needles.

The interesting point about them in Gammarus is that they are more or less rigid; for some, at any rate, of the phagocytes composing them secrete a clear yellowish chitinoid substance round the parasites. This secretion is very similar, if not identical, with the so-called ' pathological chitin ' with which wounds on the surface of the body are temporarily closed. Although the chitinoid substance gradually darkens so that the older parts of the nodules are very dark brown they remain more or less transparent. The dark nodules may be seen through the body-wall of the infected Gammarus as conspicuous black specks.

Aggregations of phagocytes may occur in almost any part of the body, frequently in the limbs and gills, where the early stages may easily be studied. There can be no doubt that the nodules are formed by the gradual accumulation of phagocytes —the newly-arrived being applied to the circumference of the others—thus giving a concentric structure with the undigested yeasts lying in vacuoles (fig. 10, Pl. 20). Quite large bodies may be formed in this way measuring up to 100μ in diameter. In sections stained by the Carmine-Claudius method, traces of the remains of the blue-stained yeasts may generally be seen even towards the darkened centre of a nodule; farther out the parasites may be sufficiently unchanged to be recognized, and pinkish remains of phagocyte nuclei may be discernible also, while on the periphery recently arrived phagocytes with their included yeasts may be easily recognized.

Similar nodules also occur amongst the viscera, especially in the phagocytic organs. Gammarids resemble Decapods among Crustacea in being supplied with special phagocytic organs (' organe phagocytaire hépatique ', Bruntz, 1907).

In Gammarus pulex the organ consists of a more or less diffuse tissue in close association with the fat body, and forms a network over and between the hepatic coeca, alimentary canal, and gonads, thus helping the mesenteries to support these organs.

The cells of the phagocytic organ vary much in size. Bruntz gives 25–45μ as the diameter of the largest. In Gammarus with recent heavy infections of yeasts, I have found that nearly all the cells of this organ may contain yeasts and be much hypertrophied, some attaining a diameter of 80μ. However, after some time, in a Gammarus that is putting up a good resistance, numerous chitinoid nodules are to be found embedded in the tissue, many cells of which may now be free from parasites. The outside healthy cells appear to have increased and compressed the cells containing yeasts into more or less compact spheres. In these concentric nodules the yeasts remain embedded in the chitinoid secretion and destroyed. The cells immediately surrounding these bodies are somewhat flattened, appearing to form one or two layers round the nodule. Throughout the phagocytic organ there are sometimes numbers of these chitinoid nodules[1] of all sizes and all shades of colour from pale yellow to very dark brown. Similar, though somewhat smaller nodules have been found in the region of Bruntz's lymphoid gland situated dorsally between the eyes, testifying to the activities of the newly-formed leucocytes.

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[1] These nodules are curiously similar to the tubercle nodules found in man, where the caseation may be due to a secretion of the human phagocytes corresponding to this secretion of a chitinoid substance in Gammarus.

As one would expect, there were far fewer chitinoid nodules in a specimen in which the disease proved fatal in about 19 days after infection than in one which fought against the infection for some months before being overcome (see Transmission Experiments, p. 347).

Similar chitinoid bodies must have been seen in many other Arthropods ; but, so far as I can discover, their nature and development has not been clearly established. Metalnikow (1920), for example, mentioned that when injected into larvae of Galleria mellonella tubercle bacilli were destroyed and transformed into pigment in what he called 'capsules'. This term surely implies some rigidity, though in a small caterpillar the presence of the chitinoid secretion might be difficult to observe.

Hollande (1920, p. 670) also said nothing of the chitinoid nature of these 'capsules', which he called 'nodules leucocytaires'. He objected to their brown colour being attributed to the decomposition of the tubercle bacilli, pointing out that they became brown when the inclusions were other bacteria or even inert substances. In a subsequent paper (1920, p. 726) he suggested that the brown pigment might be produced by a peroxydase in the leucocytes acting on the small quantity of blood plasma adhering to the body phagocyted. He mentioned that a similar colour change took place in the blood of this and some other insects when left in the presence of oxygen in the air. I have never been able to demonstrate the least sign of change of colour in Gammarus blood left in the air,[1] and, further, if this were the true explanation of the colour of the nodules, one would expect some signs of colour developed in the ordinary phagocytes such as those represented in fig. 8, Pl. 20.

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[1] Occasionally the blood is distinctly bluish while inside the Gammarus, and this purplish blue colour, due to the oxydation of the haemocyanin in the blood, is very evident when the gills are distended with it. Gammarus sometimes die with their gills in this condition and the body extended, especially when making an unsuccessful attempt to moult. In fact the blood is often so dark under these conditions in the distended gills that they appear black by transmitted light.

The ordinary 'nodules leucocytaires' of worms, &c., are also brown as a rule; but in those that I have studied (1916, p. 209) the pigment is always in definite brown granules or concretions which appear to be excretory products ingested by the phagocytes in the ordinary way and brought to the nodules in addition to parasites.

The interest attaching to the nodules of Gammarus is the presence of the chitinoid substance itself. The fact that it gradually becomes dark brown might be due to degenerative changes in the phagocyte during the secretion, for it obviously becomes necrotic.

The concentric chitinous bodies described by Alverdes (1912) in some sections of Branchipus would seem to be very similar to the nodules in Gammarus. They are said to occur in the fat cells of Branchipus; but those drawn show no sign of having contained oil globules, and I can only think that owing to close proximity of the two tissues the author may, in the sections (apparently the only material available to him), have mistaken the nature of the cells infested—he also failed to find any definite parasites.

PHAGOCYTOSIS IN (2) MICROSPORIDIAL DISEASES: RARE FORMATION OF CHITINOID NODULES CONTAINING SPORES.

It has been pointed out before that protozoa unless in cysts or spores are very seldom, if ever, interfered with by phagocytes (Goodrich and Pixell Goodrich, 1919). In Gammarus infected with microsporidial diseases small chitinoid nodules have very occasionally been seen, the contents being apparently a few spores.

Some other instances of phagocytosis of unprotected microsporidial spores have been reported since Metchnikoff (1892) stated that such spores were attacked to a slight extent in Daphnia. As a rule, this stage of a microsporidian parasite is protected by being produced inside a cell or mass of tissue of the host. Early stages (merozoites) may swarm in the blood without being touched by the phagocytes; but when about to form spores, the sporonts of both Thelohania and Nosema establish themselves within a host muscle between its contractile fibrils (fig. 1, Pl. 20). In this position the spores remain, as a rule, apparently causing very little inconvenience to the host, until liberated by its death. Their presence may be detected macroscopically by a certain opacity of the infected muscles. On examination of a piece of the muscle microscopically the single spores may be easily made out in the case of Nosema infection (fig. 1, Pl. 20) or the groups of pansporoblasts each containing eight spores in Thelohania.

Among numerous specimens of Gammarus infected with microsporidial spores, I have had two cases of Nosema infection and one of Thelohania in which several muscles were distinctly brownish, giving a very peculiar brown and white striped appearance to the animal. Upon examination after death each of the brown streaks was found to owe its colour to chitinoid nodules of various shades of yellow to deep brown, more or less projecting from its surface. On further examination by section and teasing the whole streak was found to be the remains of a muscle-fibre which had become literally a mass of spores owing to the breakdown of the contractile fibrils which separate these spores in ordinary infections. Further, the whole necrotic muscle had lost all connexion with surrounding tissues and was lying quite free in the body-cavity and was being attacked by phagocytes. The microsporidial spores, no longer protected by living host tissue, were being rapidly ingested by phagocytes attacking the whole surface of the dead muscle. As usual the phagocytes were collecting into nodular masses and, owing to the secretion of the usual chitinoid substance, the regions where the attack was most advanced had the darkest brown colour. It was, of course, these dark chitinoid nodules on the surface of the necrotic muscle that gave it the strange brownish colour.

Each nodule contained spores in various stages of destruction embedded in an amorphous substance of a brownish colour. The largest nodules containing the greatest number of spores were generally the darkest brown. The mass of spores towards the centre of the muscle was still colourless and apparently unchanged in any way.

There can be no doubt, I think, that these elongated masses represent muscles which have been overrun by sporonts to a greater extent than usual, so that practically no contractile substance remains and the whole muscle is exhausted and killed during the formation of the spores. Its destruction by phagocytes is then just what would be expected, and the spores, no longer protected by the tissue of the host, are incidentally destroyed at the same time.

Zwölfer (1926) described a similar condition of some of the muscles in specimens of Gammarus pulex infected with the microsporidian Plistophora blochmanni. Apparently he only studied this 'abnormal condition' in sections, and its true nature in these is more difficult to elucidate. Consequently, although he suggested that the host's connective tissue was responsible, he did not definitely commit himself to the view that the brownish 'Kittsubstanz', in which the more or less digested spores are found embedded, might be produced by phagocytes.

However, Zwölfer pointed out that there was possibly something of the same character to account for the so-called 'black spores' sometimes found in malarial infection of flies which Brug (1916) attributed to chitinizing by the host of the cyst of the parasite. In the light of these observations on Gammarus there can be little doubt that these malarial cysts owe their dark colour to having been attached by the hosts' secreting phagocytes. The cysts normally remain covered by the layer of mesoblastic epithelium covering the fly's alimentary canal. As they grow, the cysts push out this layer protecting them into the haemocoele; possibly it is owing to the rupture of the mesoblastic epithelial cells at some point that the host's phagocytes are enabled to attack the cyst and secrete the chitinoid substance which gradually darkens. The case would then be similar to Metchnikoff's historic account of a nematode in Lumbricus mentioned below, p. 343.

The other scattered references to black spores or cysts, some of which were mentioned by Zwölfer, can probably be explained in a similar way.

3. Phagocytosis of Foreign Bodies: Formation of Chitinoid Nodules containing Indian Ink.

When first I studied the production of these chitinoid bodies in Gammarus infected with yeast disease, it seemed possible that the chitinoid substance might be some product of the digestion of the yeasts. Even after coming across the above described instances of the occurrence of a similar chitinoid substance in phagocytes digesting microsporidial spores, it still seemed possible that it was derived from the bodies being digested. This idea was further encouraged by the fact that Bruntz, who has written three papers on phagocytosis in Crustacea including Gammarus, does not once mention these chitinoid nodules (Bruntz, 1907, where references will be found to his earlier works).

To prove that the chitinoid substance in the nodules is not a product of the digestion of the yeasts or microsporidial spores, I injected many Gammarus (free from diseases caused by these organisms) with ammonium carminate and Indian ink (Cuénot's method, 1896).

For this and other injections it has been found best to use a micropipette drawn out to a very fine point at one end and at the other to attach a long narrow rubber tube which can be held in the mouth. The best region for injection of Gammarus is ventrally between the sterna into the haemocoelomic cavity about the middle of the body.

Many cells of the phagocytic organ have been found crowded with ink particles even after five hours. In one Gammarus killed and mounted whole within 24 hours after injection, the carmine could be seen being excreted by the head-gland, which was deeply stained red; and the finer particles of ink had been carried by the blood all over the body and were specially numerous in the extremities of the smaller appendages. In one pleopod small aggregations of phagocytes with ingested ink particles were very distinct. Definite chitinoid nodules were present in a specimen which died 9 days after injection, and in one, which died after 19 days, they were very numerous. Another injected specimen died 30 days afterwards while trying to moult, and in this there were hundreds of chitinoid nodules specially conspicuous in the pleopods and maxillipeds. Another died under much the same circumstances 49 days after injection, and its numerous nodules also contained ink particles and a few fine carmine granules or remains of cells stained with carmine.

Finally, a specimen injected on February 6 was alive and apparently quite healthy on May 24, when it was killed and dissected and found to contain no nodules. This Gammarus had moulted twice since March 30, when a few nodules had been recognized inside some of the smaller appendages, so I think it may be concluded that the nodules are sometimes eliminated. Although one suspects that chitinoid nodules often work their way out through the tissues and are thrown off with the moult, I have no really direct evidence of the fact. Freshly cast moults, frequently examined for one thing or another, have never revealed loose nodules inside them. On one occasion I found three nodules in the tip of an appendage left in a moult. Sometimes it has been difficult to make out whether a dark speck on the moult is a cicatrice or a small nodule stuck to the inner side of the chitin.

The fact that Bruntz appears to have entirely missed the formation of chitinoid nodules can only, I think, be explained by supposing that all the specimens examined by him were healthy, and that those which he injected with Indian ink must have died or been killed too soon after injection for the nodules to have formed.

THE CHITINOID SECRETION OF THE PHAGOCYTES.

This is not the first time that phagocytes have been suspected of secreting chitin. Claparède (1868) described bodies which he thought looked like chitinized rodlets in the leucocytes of Ophelia. They were quite insoluble in acetic acid and also in dilute and concentrated nitric acid. Shaeppi (1894), who studied them in greater detail, considered these rodlets to be chitin, and was convinced by Eisig that they were at the same time excretory products like the deposits found by the latter (1887, p. 689) in some leucocytes of Capitellids. Metchnikoff (1892, figs. 4 and 5) assumed that the ' cyst ' that he found surrounding a nematode which was being attacked by the phagocytes of Lumbricus had been secreted by the nematode as a protection. Cuénot, however, in one of his later papers (1898) on this subject came to the conclusion that in Oligochaetes it was the phagocytes which secreted the amorphous cyst round the parasitic nematode and caused its destruction by isolating it from its host, and he described similar ' cysts ' secreted by phagocytes round pointed objects such as setae. Nowhere, however, so far as I can find, has he made any suggestion as to the nature of these ' amorphous cysts '.

MICROCHEMICAL TESTS AND STAINS.

The amorphous yellow-brown substance secreted by the phagocytes of Gammarus looks very like chitin and gives the same solubility tests, that is to say, it is insoluble in caustic potash (30–40 per cent.) even when heated and left in the solution for days, and is also insoluble in dilute acids.

Schulze's reaction had to be tried many times with freshly prepared solutions before I could convince myself that the chitinoid substance of the nodules did not give the ordinary chitin test. The difficulty was increased by the nodules themselves being already dark brown when they had attained any size. It was not possible to test sections, for the fixatives (Schaudinn and Bouin as well as ordinary formalin) were found to have so changed the real chitin of the exoskeleton that it was impossible to get the pinkish-violet reaction with that either.

There was the same difficulty with regard to sections in carrying out Bethe's reaction, but the nodules and pathological chitin were shown to be different from true chitin by testing parts of a Gammarus containing them after macerating in the mixture of alcohol and nitric acid for some days.

With nigrosin and also with Mallory's stain there was a very distinct difference between the substances in thin sections of

Gammarus. The true chitin stained pale blue as usual with the former and bright blue with the latter in sections which had been fixed in a fixative containing mercuric chloride, whereas the chitinoid secretion of the phagocytes remained quite unchanged in colour. We may therefore conclude that the chitinoid secretion of the phagocytes is not identical with true chitin.

THE INFECTING ORGANISM—CRYPTOCOCCUS GAMMARI VEJD.

The yeast which causes this disease in Gammarus pulex multiplies rapidly by budding (figs. 2 *a*, 3 *a*, Pl. 20), but the buds do not remain attached for long, i. e. no strings of parasites are formed. No ascospores nor other reproductive forms have been found either in the blood or on artificial media. We may conclude, therefore, that this organism belongs to the genus Cryptococcus Kützung-Vuillemin, which Guilliermond (1912, p. 295) describes as 'levures sans asques, parasites des animaux'. This author records several species of the genus; but they have not much resemblance to the Gammarus parasite—the closest being Cryptococcus parasitaris (1912, p. 483), which, he says, Trabut discovered 'sur les sauterelles (Acridium peregrinum)' as rounded cells (3–4μ). Quéquen (1902), to whom he refers, gives no further information of importance. However, the specific name of the Gammarus parasite seems to be already fixed, for it has apparently been seen before and mistaken for a bacterium.

In 1900 Vejdovsky described a parasite from a species of Gammarus, which he called Gammarus zschokkei, sent to him by Professor Zschokke from Lake Garschina, Switzerland. Of 300 specimens received in alcohol, he said that 75 per cent. were infected. In his first paper he was uncertain about the status of the parasite, pointing out that, as its life-history was not wholly known, he was leaving the bacterium, as he called it, unnamed. It seems extraordinary that he should have missed its reproduction by gemmation, for he described and figured young forms both round and oval and his figure (3 *h*) might be a distorted budding form. Of course, gemmation is much more obvious in living parasites or in those well fixed, and to the want of such material was no doubt partly due this curious mistake. In his second paper (1904) Vejdovsky called the parasite Bacterium gammari, and he gave more figures and had a great deal to say about the nucleus and the significance of its presence in a bacterium! His assistant, Mencl (1907), re-stained some of the preparations, but also missed the gemmation, though his figures again could hardly be mistaken for another organism. The long rod-shaped forms reappear as well as the oval and minute round ones; also his figures 8 and 19 possibly represent budding forms.

We may therefore conclude that the correct name of this parasitic yeast is Cryptococcus gammari Vejd., 1904.

The so-called bacterial infection which Tait (1917) described in Gammarus marinus was probably due to the same or a very similar yeast. In March 1925 I found what appeared to be the same parasite, Cryptococcus gammari, in a few specimens of the brackish-water species, Gammarus duebeni, from Liverpool. It is impossible to be certain of the identity of these forms for I have not been able to obtain them in pure culture, and so have not tested their action towards the sugars, &c. Cultivation has often been attempted on various media, especially Sabouraud, Musgrave and Clegg, and beer-wort, without success. Once or twice when a growth was obtained, contamination was suspected, and once definitely proved, as will be described below.

In making preparations of phagocytic tissue from the walls of the alimentary canal of one Gammarus I came across some rather similar yeast-like bodies growing out into mycelial threads. These appear likely to belong to an entirely different fungus, possibly one merely passing through the alimentary canal.

An Eccrinid, possibly closely related to that described by Leger and Duboscq (1911), Poisson (1927), is nearly always to be found in the proctodeum after being thrown off in the Gammarus moult.

Another fungus found in an Amphipod (Talitrus locusta) by Hermann and Canu (1891) is said to grow easily in artificial culture, and to be closely related to Sacchromyces albicans (Oidium).

Hollande (1919) briefly described a yeast-like organism in the blood of an Orthopteran (Caloptenus), and said that another had also been recorded in the blood of Cochylis caterpillars. In 1922 Hollande and Moreau described another form living in the blood of grasshoppers (Stenobothrus, four species) in France. The appearance of the latter parasite in the blood of its hosts seems to be exactly like that of the yeast described here from Gammarus. However, the authors said that in artificial culture it grew out into a mycelium with conidia, and they called it Isaria stenobothri n. sp.

Perhaps I should mention here that at one time I frequently obtained a very similar, if not identical, growth in my cultures ; but becoming suspicious of the ease with which it grew, I examined the Ringer's solution that was supposed to be sterile and that I had been using to dilute the blood and obtained a prodigious growth of the mycelium and conidia from that alone! I am now quite satisfied that the organism which causes the disease in Gammarus pulex is a yeast which multiplies by gemmation only.

TRANSMISSION OF THE YEAST DISEASE.

The yeasts undoubtedly remain alive and infective for a considerable time in the Gammarus corpses left in water, though they do not continue to multiply. In time they are eaten by various aquatic animals, even Paramoecium has been found at times with numerous food vacuoles full of the partially digested yeasts.

The infection of fresh hosts is not, as a rule, by the mouth and alimentary canal, as described by Hollande (1919) for Stenobothrus, but directly into the blood through wounds, especially during the moulting period. Gammarus, like other Arthropoda, frequently loses its appendages by accident, and, at moulting, injuries are of course very easily produced, so that opportunities for infection are numerous.

When hungry a Gammarus readily eats a dead or moribund specimen even when crowded with yeasts. However, by dissecting those that have so fed or by examination of sections, I have obtained no evidence that the yeasts multiply in the alimentary canal of their host. Occasional yeasts may be found in the mid-gut; but they appear to be digested and to pass no farther. No parasites have ever been seen inside intestinal cells.

INFECTION EXPERIMENTS.

Experiments have been undertaken with Gammarus from Albury, a locality from which no cases of yeast disease have ever appeared.

The specimens used for experiment were kept in glass bowls about four inches in diameter containing tap-water to a depth of about one and a half inches, also some 'American weed' to which the Gammarus like to cling. Each Gammarus was kept singly in a bowl in order not to introduce the possibility of their injuring each other. To expose them to infection, the corpse or part of the corpse of an infected specimen was given. This they ate readily, when no other food was present, and in doing so scattered yeasts in the water around them and swallowed many also.

Series I.—On several occasions Gammarus kept under observation while exposed to infection have been found to remain healthy until after they have moulted, and the disease has then shown itself firmly established in 19–20 days and proved fatal a few days later. There can be little doubt that the yeasts have gained entrance to the blood through some wound made at the time of moulting.

Series II.—Two Albury Gammarus safely accomplished their moult during the night of February 18, 1926. On the next day they were exposed under identical conditions to the same infection, i.e. they were each given half a specimen full of yeasts. The tips of two legs of one Gammarus were, however, snipped and the other Gammarus left intact. Both were seen to eat some of the corpse full of yeasts on February 19.

On March 11, i. e. after 19 days, the stumps of the two snipped legs were found to be opaque and badly infected with yeasts. On the 15th the whole Gammarus was swarming with yeasts and killed for fixation and sections. The Gammarus which had not been cut but had fed on the yeasts from the same corpse was perfectly healthy and remained so for a further four months under observation and was then returned to a bowl of stock specimens.

Similar experiments of this kind were not always successful, for both Gammarus sometimes remained healthy; but this, I think, may be attributed to the protective action of their phagocytes. An uninjured Gammarus has never been known to become infected from feeding on the yeasts.

Series III.—Artificial infection by the prick of a needle steeped in the yeasts has also been effected but not very often; it is generally necessary to inject a considerable amount of emulsion of the yeasts to be sure of success.

Of eight Albury Gammarus inoculated in this way on January 31 only two died of the disease—one in trying to moult on March 2; the other moulted successfully twice, but on May 14, when another moult was due, it showed symptoms of the disease and succumbed a few days later.

The long-continued activity of the host's phagocytes was shown by the much larger number of chitinoid nodules in the latter specimen than in the former.

AUTOTOMY FOR THE CURE OF YEAST DISEASE.

In addition to phagocytosis, Gammarus sometimes resorts to autotomy in striving to rid itself of yeasts. It has often been noticed that when Gammarus has been infected by injury to the tip of an appendage, the stump of the injured appendage, be it a pleopod, uropod, antenna, or peraeopod, is always the most severely infected and becomes so crammed with parasites that the circulation is practically stopped. In fact Gammarus are sometimes found with one or two opaque appendages containing numerous yeasts, while the rest of the body is fairly free from them ; further, these infected appendages are often in process of being thrown off autotomously. Voluntary amputation can apparently take place at any joint of an appendage across which a plate of the usual yellowish-brown chitinoid substance is secreted by the phagocytes collected in the region. Meanwhile the distal part of the appendage in which the circulation of blood is stopped becomes densely opaque and in the end withers and falls off, leaving the chitinoid partition to form the usual dark cap to the stump as described for the healing of a cut leg.

The accumulation of parasites in the appendages is generally, no doubt, the result of local infection. Such appendages would show black tips or other scars, unless the site of infection had been a small wound of which all outward signs had been lost by a subsequent moult. Occasionally, however, the presence in a Gammarus of several opaque appendages cannot be explained as repeated local infections ; there does, in fact, seem to be a distinct tendency on the host's part to restrict its yeasts to the tips of its appendages. This cannot, I think, be in any way accounted for by gravity, for Gammarus very often support themselves upside down by clinging to weed in their bowls. It is more likely to be due to the small size of the blood spaces in the limbs making it possible to filter out the yeasts, as it were, from the blood during its slow circulation down one side and up the other : communication with the main blood flow would be more or less blocked during this process by accumulations of leucocytes.

Anyhow, this attempt to restrict the infection and deal with it locally by autotomy does not appear to be very successful, for dead and dying specimens may be found with the process in progress. Perhaps autotomy in Gammarus may be looked upon as a policy of desperation.

It does not appear to be carried out rapidly enough to stop the spread of infection, though it is certainly quicker than dealing with the invading organisms in chitinoid nodules, which have never been found in legs severed autotomously.

The occurrence of Gammarus with opaque appendages, whether they are being amputated or not, is a fairly sure sign of yeast disease. If in addition the specimen is speckled showing the presence of scattered chitinoid nodules, yeast disease may be safely diagnosed without waiting to confirm the diagnosis by microscopic examination of the blood to find the yeasts.

I have seen no special necrosis of the muscles suggesting that there is any definite muscular intoxication as described by Hollande and Moreau (1922) as the cause of death in Stenobothrus infected with a similar parasite.

In Gammarus, when the infection cannot be dealt with locally, the yeasts circulate with the blood all over the body, the circulation becoming more and more sluggish as the parasites increase. Finally the aorta and its branches become distended and apparently blocked with parasites and necrotic phagocytes containing them, and it is this that appears to be the prelude of death.

SUMMARY.

1. A yeast, Cryptococcus gammari (described by Vejdovsky, 1900, and others as a Bacterium) causes a disease in Gammarus which may be lethal, but is sometimes cured by phagocytosis and autotomy.

2. The phagocytes most effective in destroying parasites secrete a chitinoid substance in which to imprison them.

3. By co-operation of the phagocytes large chitinoid nodules may be formed and subsequently become dark brown. They then appear as black specks through the body-wall of the Gammarus.

4. The spores of Nosema and also those of Thelohania occasionally destroy a muscle-fibre of Gammarus. This fibre may then be attacked by phagocytes which secrete the chitinoid substance round the spores and destroy them.

5. A similar chitinoid substance (black or pathological chitin) by which wounds are closed in Gammarus is also a product of its leucocytes.

6. The chitinoid substance produced by the leucocytes of

Gammarus is not chemically identical with the true chitin secreted by the epidermis.

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REFERENCES.

Alverdes, F. (1912).—" Über konzentrisch geschichtete Chitinkörper bei Branchipus grubii ", ' Zool. Anz.', xl, p. 317.

Brug, S. L. (1916).—" Die schwarzen Sporen (' black spores ') bei der Malariainfektion im Mückenkörper ", ' Arch. Protistenk.' 36, p. 188.

Bruntz, L. (1907).—" Organes lymphoïdes, phagocytaires et excréteurs des Crustacés supérieurs ", ' Arch. Zool. Expér.', 4. vii, p. 1.

Claparède, E. (1868).—' Annélides, Chétopodes du Golfe de Naples.' Geneva.

Cuénot, L. (1896).—" Études physiologiques sur les Orthoptères ", ' Arch. Biol.', xiv, pp. 293–316.

—— (1898).—" Études physiologiques sur les Oligochètes ", ' Arch. Biol.', xv, p. 114.

Eisig, H. (1887).—"Monographie der Capitelliden des Golfes von Neapel", ' Fauna und Flora des Golfes von Neapel ', xvi.

Goodrich, E. S. (1919).—" Pseudopodia of the Leucocytes of Invertebrates ", ' Quart. Journ. Micr. Sci.', 64, p. 19.

Goodrich, E. S., and Pixell Goodrich, H. (1919).—" Leucocytes and Protozoa ", ' Contributions dedicated to Sir Wm. Osler on his Seventieth Birthday ', p. 958. New York.

Guéquen, F. (1902).—"Les Champignons parasites de l'homme et des animaux", ' Thèse agrég. Pharmacie.' Paris.

Guilliermond, A. (1912).—' Les Levures.' Doin, Paris.

Hermann, G., and Canu, E. (1891).—" Champignon parasite du Talitre ", ' C.R. Soc. Biol.', sér. 9, 3, p. 646.

Hollande, A.-Ch. (1919).—" Formes-levures pathogènes observées dans le sang d'Acridium (Caloptenus) italicus L.", ' C.R. Ac. Sc.', 168, p. 1314.

—— (1920).—" La Formation du pigment brun-noir (mélanine) au cours de la phagocytose chez les Insectes ", ' C.R. Soc. Biol.', 83, p. 726 ; see also p. 670.

—— (1921).—" La Cellule péricardiale des Insectes ", ' Arch. d'Anat. Micros.', xviii, p. 85.

Hollande, A.-Ch., and Moreau, F. (1922).—" Présence de formes-levures bourgeonnantes dans le sang des Stenobothrus, etc.", ' Arch. Zool. Expér.', 61, Notes et Revue, 1922, pp. 59–74.

Léger, L., and Duboscq, O. (1911).—" Sur les Eccrinides des Crustacés Décapodes ", ' Ann. de l'Univ. de Grenoble ', xxiii, p. 139.

Léger, L., and Hesse, E. (1917).—" Sur les microsporidies de la crevette d'eau douce ", ' C.R. Soc. Biol.', 80, p. 12.

Mencl, Em. (1907).—" Nachträge zu den Strukturverhältnissen von Bacterium gammari Vejd.", 'Arch. Protistenk.', viii, p. 259.

Metalnikow, S. (1920).—" Sur la digestion des Bacillus tuberculeux dans le corps des Chenilles des Mites des Abeilles (Galleria mellonella) ", ' C.R. Soc. Biol.', 83, p. 214.

Metchnikoff, E. (1892).—' L'Inflammation.' Paris.

Pantel, J. (1910).—" Caractères parasitiques aux points de vue biologique, éthologique et histologique ", ' La Cellule ', xxvi, p. 165.

Pixell-Goodrich, H. (1916).—" Gregarines of Glycera ", ' Quart. Journ. Micr. Sci.', 61, p. 205.

Poisson, R. (1927).—" Sur une eccrinide nouvelle, . . . du rectum de l'Orchestia bottae ", ' C.R. Acad. Sc.', 185, p. 1328.

Shaeppi, T. (1894).—" Das Chlorogogen von Ophelia radiata ", ' Jen. Zeit. f. Naturwiss.', xxviii, p. 247.

Stempell, W. (1902).—" Ueber Thelohania mülleri ", ' Zool. Jahrbuch Anat.', xvi, p. 235.

Tait, J. (1908).—" A simple method of observing the agglutination of the blood corpuscles in Gammarus marinus ", ' Quart. Journ. Exper. Physiol.', 1, pp. 247–9.

—— (1910).—" Blood Coagulation in the amphipod Gammarus ", ' Journ. Physiol.', xl, p. xli.

—— 1917).—" A Bacterial Infection of Gammarus ", ' Proc. R. Phys. Soc. Edin.', xx, p. 159.

Vejdovsky, F. (1900).—" Bemerkungen über Bau und Entwickelung der Bakterien ", ' Centralbl. Bakteriologie ', Abt. 2, vi, p. 577.

—— (1904).—" Ueber den Kern der Bakterien und seine Teilung ", ibid., xi, p. 481.

Winterstein, H. (1925).—'Handbuch der Vergleichenden Physiologie', i, 1, p. 720.

Zwölfer, W. (1926).—" Plistophora blochmanni, eine neue Microsporidie aus Gammarus pulex ", ' Arch. Protistenk.', 54, p. 261.

EXPLANATION OF PLATE 20.

Illustrating Mrs. Goodrich's paper on Reactions of Gammarus to Injury and Disease.

REFERENCE LETTERS.

cn, chitinoid nodule; *cs*, chitinoid substance; *dc*, darkened chitin; *e*, epithelial cells; *N*, nuclei; *n*, flattened necrotic nuclei of phagocyte; *p*, phagocytes; *t*, terminal fragment of chitin; *y*, yeasts.

Fig. 1.—Spores of Nosema lying between the fibrellae of a piece of teased muscle. Fresh preparation. × 1,000.

Fig. 2.—Yeasts stained intra-vitam with neutral red (*a*) oval form with minute bud, (*b*) rod-shaped form. × 3,000.

Fig. 3.—Yeasts showing vacuoles and chromatic bodies. Schaudinn, iron haematoxylin. × 3,000.

Fig. 4.—Stump of limb with black cap several days after amputation. Whole preparation. × 10.

Fig. 5.—Longitudinal section through the distal end of a leg 21 hours after snipping off its tip. Schaudinn, iron haematoxylin, picro-nigrosin. × 300.

Fig. 6.—Part of section enclosed in square on left of fig. 5. × 1,250.

Fig. 7, *a* and *b*.—Phagocytes with recently ingested yeasts. Fresh preparation. × 2,000.

Fig. 8, *a* and *b*.—Stages in the destruction of a single phagocyte by engulfed yeasts showing necrotic nuclei (N). Schaudinn, iron haematoxylin. × 2,000.

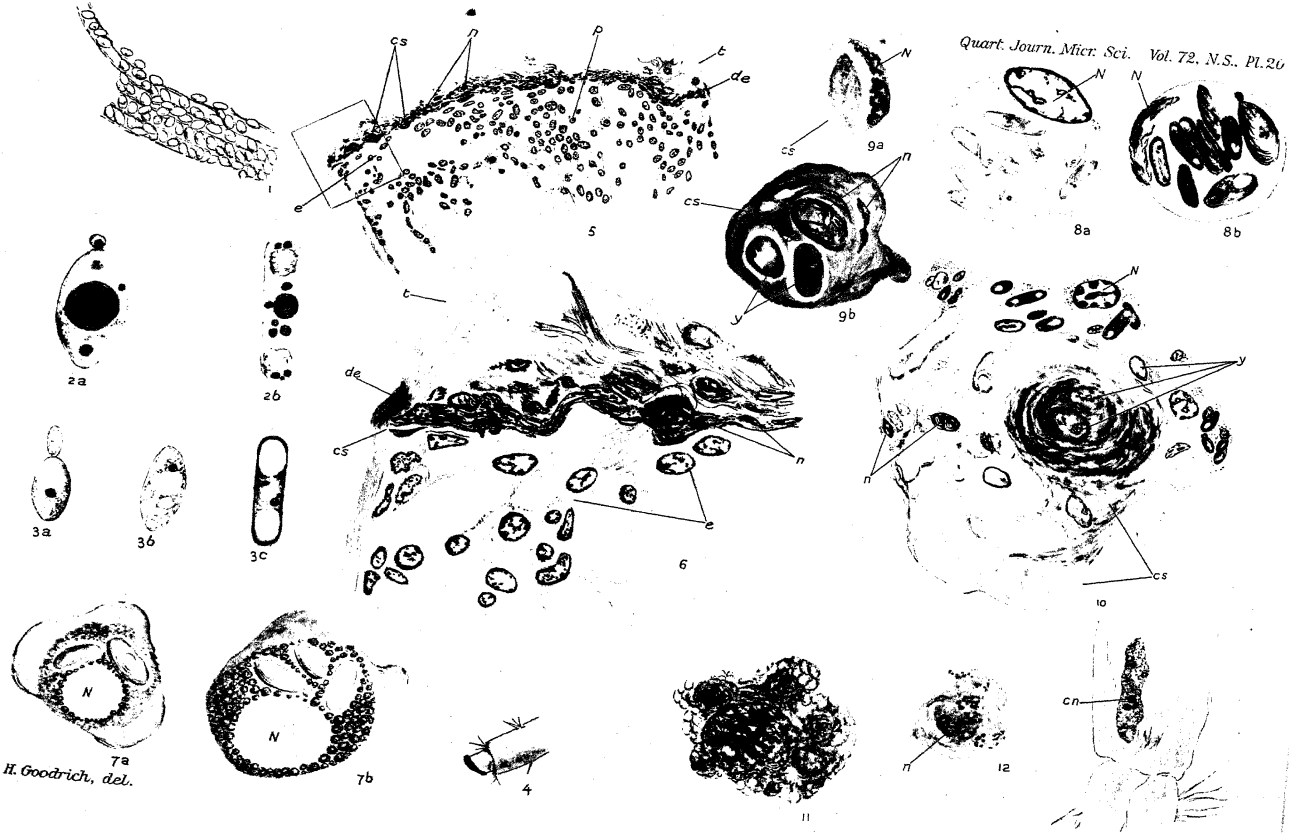
Fig. 9.—Sections of early stages in nodule formation: (*a*) a single phagocyte embedding a single yeast; in (*b*) at least two phagocytes have been involved. Schaudinn, Carmine-Claudius. × 2,000.

Fig. 10.—Section through a fairly large chitinoid nodule showing remains of engulfed yeasts towards centre and others being brought to the outer side by fresh phagocytes. Schaudinn, Carmine-Claudius. × 1,500.

Fig. 11.—Surface view of chitinoid nodule consisting of several necrotic phagocytes crowded with spores of Nosema from a dead muscle. Fresh preparation. × 1,000.

Fig. 12.—Small nodule containing particles of Indian ink. Fresh preparation. × 1,000.

Fig. 13.—Part of a pleopod, showing the destruction by phagocytes of a small necrotic muscle full of spores of Nosema. Fresh preparation. × 50.



H. Goodrich, del.

A species of Proboscidiella from Kalotermes (Cryptotermes) dudleyi Banks, a termite of Central America, with remarks on the oxymonad flagellates.

By

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With Plates 21–24.

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I. INTRODUCTION AND HISTORICAL ACCOUNT.

AN interesting group of Protozoa living in termites is that comprising the oxymonads, which in many ways are very different from other known flagellates. By prolongations of the anterior ends of the bodies they may attach themselves to the lining of the intestine, but they are also able to swim about in the lumen of the canal. Having no cytostomes they ingest wood, as do many termite flagellates, through the general surface of the body.

What was probably the first member of this group to be recorded is Microrhopalodina enflata from Kalotermes flavicollis of Europe, which was described but not figured by Grassi and Foa (1911). In the usual form this organism is multinucleate, and is attached to the intestinal wall by a 'peduncle' or 'neck'. When the host moults, however, the parasite is said to become free, lose the 'neck', and develop flagella. A fuller discussion of this genus will be given later (p. 378).

Janicki (1915) described Oxymonas granulosa from Neotermes connexus ('Kalotermus castaneus') of Hawaii, giving an account of mitotic division. He noticed that this organism resembled Microrhopalodina in several respects, but separated it generically chiefly because, in contrast to Grassi's flagellate, it was usually uninucleate, though rarely binucleate.

In his monograph on the flagellates of termites Grassi (1917) figures flask-shaped flagellates from N. erytraeus and Glyptotermes parvulus which are undoubtedly oxymonads. He did not recognize that fact, however, but supposed them to be developmental stages of multinucleate flagellates of the family Calonymphidae.

Recently Kofoid and Swezy (1926 *a*, *b*) gave an account of three new species of Oxymonas, re-examined Janicki's species, correcting his account for flagella and other structural details, and described a new multinucleate oxymonad, Proboscidiella multinucleata. Later in the same year a small species of Oxymonas was recorded from Cryptotermes hermsi (Kirby, 1926).

As a result of the study of the Protozoa of termites collected in 1925 in the Canal Zone and Costa Rica, as well as of several species obtained from other localities, it is possible for the writer to make some additions to the knowledge of this group of flagellates. These studies, as well as others still unpublished, have been facilitated by material placed at my disposal by Dr. A. E. Emerson, Dr. S. F. Light, Mr. G. F. Hill, Mr. J. E. Zetek, and Dr. L. R. Cleveland. The Central American collections were made with the aid of a grant given to Dr. L. R. Cleveland by the Bache Fund of the National Academy of Sciences, and were identified by Dr. T. E. Snyder. Many of the illustrations were prepared by Miss Lisbeth Krause.

II. Material and Technique.

The observations on living uninucleate oxymonads which are here recorded were made on material from four species of Kalotermes s. str., two of which came from Central America and two from California. Of C. dudleyi the material at my disposal was obtained from two sources: a colony (T–234) collected by Mr. Zetek in Ancon, and one (T–239) collected from furniture in Balboa, Canal Zone. Smears from the former were prepared at the Barro Colorado Island Biological Station soon after their capture; the latter were brought to New Haven and fed for variable periods on filter paper before preparations were made.

In addition to the usual methods of preparing material, fixation in osmic vapour and use of the dark-field condenser were of much assistance. The former procedure, with subsequent staining in iron haematoxylin, facilitated observations on the flagella and blepharoplasts. Unfortunately this method was not used for Proboscidiella, but fixation in Flemming's fluid without acetic acid gave comparable results. Staining in Delafield's haematoxylin after fixation in Schaudinn's fluid was very satisfactory for nuclear structure and assisted in the formation of conclusions concerning the existence of a parabasal body, for in other material on the same slides (Devescovina, Stephanonympha, and others) the parabasal was well stained.[1] The dark-field condenser fitted to a mon-objective binocular microscope proved very helpful in determination of the number and length of flagella in the uninucleate oxymonads, but this equipment was obtained too late for use with Proboscidiella. On serial sections of the intestine of C. dudleyi the flagellates could be studied in their normal relation to the intestinal wall.

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[1] The parabasal bodies of many flagellates in termites, including Trichomonas, Devescovina, Calonympha, and Stephano-

III. THE UNINUCLEATE OXYMONADS.

The oxymonads have a limited distribution among termites, as they have been found only in members of one sub-family, the Kalotermitinae. In this group, however, they seem to be widespread, for an oxymonad occurs in thirty-one of the thirty-six species listed on pp. 382–3, and is not known to be absent from four of the other five. Although none has been found by the writer in Porotermes grandis, the significance of this probable exception is less because the genus Porotermes differs both structurally and in flagellate fauna from other members of the sub-family to a greater extent than these differ among themselves. In twenty-two species of these termites which the author has studied, and in six other species recorded in the literature, an oxymonad occurs which is predominantly uninucleate. In C. dudleyi this is replaced by a multinucleate form similar to P. multinucleata, and in Calcaritermes brevicollis there is, in addition to the uninucleate form, an undescribed multinucleate flagellate with an organ of attachment similar to that of Proboscidiella, but with a different form and arrangement of nuclei and body-fibres.

Observations on flagellates similar to Oxymonas from K. tabogae and K. marginipennis of Central America, and K. hubbardi and K. minor of California, were made by reflected light from the dark-field condenser. In every instance four long flagella were seen (figs. 1–9, Pl. 21). In a crushed speci-

nympha, are clearly demonstrated by staining in Delafield's haematoxylin, after fixation in Schaudinn's fluid with or without acetic acid. In the case of Metadevescovina debilis from Kalotermes hubbardi the addition of 10 per cent. acetic acid to the fixing fluid had no apparent effect on the parabasal body.

men (fig. 7) the flagella could be traced to the two blepharoplasts, from which they originated in pairs. Although individuals varied very much in length, the flagella were in all cases longer and in some more than twice as long as the body. As seen in living material, illuminated by light through the dark-field condenser, they give no impression of weakness, but on the contrary are stout and vigorous, slowly propelling organisms with anterior processes as well as others without those structures. The same number and arrangement of flagella has been determined in material from K. hubbardi and K. marginipennis fixed in osmic vapour.

In three species of Oxymonas from South American termites, and presumably also in O. granulosa, which was re-examined, Kofoid and Swezy (1926) found six short flagella, three attached to each blepharoplast. They give this number as characteristic of the genus, stating that 'this genus is fundamentally of the triflagellate type'. In accordance with this and other observations, the genus Oxymonas Janicki, 1915, is emended by them to include the two blepharoplasts, each connected by a rhizoplast to a centrosome on the nuclear membrane, and connected together by a semicircular filament, and the three flagella arising from each blepharoplast.

In features other than the flagella, the uninucleate oxymonads which the writer has observed resemble those described by Kofoid and Swezy. Because of the difference in that respect, however, it may be necessary, in agreement with the procedure followed in the case of trichomonad and other flagellates, to establish another genus for the forms with four flagella. The writer is not prepared to do this without making observations on the type species of the genus Oxymonas. It is, however, desirable to consider the quadriflagellate forms for the sake of comparing them with the new species of Proboscidiella and defining the characters of the family to which the oxymonads belong.

In addition to the value of dark-field illumination for observations on the flagella, this method facilitates study of the micro-organisms adhering to the bodies of the oxymonads as well as other flagellates of termites. The possible error, which has often been made, of confusing those organisms with flagella is thus eliminated, for their appearance and movements, when observed in this manner, are seen to be very different from those of flagella. Most of them are spiral forms, probably spirochaetes (Cleveland, 1928). By feeding the termites for a period of about ten days on filter paper soaked in 5 per cent. acid fuchsin, as recommended by Cleveland (1928), the spirochaetes may be caused to disappear from the bodies of the flagellates, as well as from the intestine of the termite. After this method had been used upon the oxymonad from K. hubbardi, rod-like forms, which had not been noticed on untreated flagellates, were found to be present.

The abundance of micro-organisms on the bodies of different individuals is, of course, very variable. The numbers vary on some species from a condition in which they cover both the body proper and the organ of attachment, to one in which there are very few or none at all. But it is possible, nevertheless, in some cases, to use the presence and abundance of certain types of micro-organisms as a specific characteristic. Thus on the oxymonad from K. hubbardi there are usually some, but not many, spirochaetes, while on that from K. panamae every individual has a dense coat of those organisms. O. pediculosa, according to Kofoid and Swezy, has a similarly large number. This point is admirably illustrated by Metadevescovina debilis from K. hubbardi, though that is not an oxymonad. On every individual of this species there are, normally, large numbers of spirochaetes, which are shorter and stouter than those on Oxymonas, forming a dense covering which has been mistaken for a coat of cilia. By feeding the host on acid fuchsin all these may be removed. On various species of uninucleate oxymonads, spirochaetes of one type, a long, narrow, much spiralled form, have been seen. As will be discussed later, this is replaced on the new species of Proboscidiella by a short, rod-like micro-organism, though longer forms are apparently present on the type species of that genus.

The above remarks on uninucleate oxymonads are included here chiefly for comparative purposes. A detailed cytological study of the structure and mitotic division, for which material from several species is available, may be expected to clarify our knowledge of the morphology and life-history of these interesting flagellates.

IV. PROBOSCIDIELLA KOFOIDI, SP. NOV.

A multinucleate oxymonad was described by Kofoid and Swezy (1926*b*) from Planocryptotermes nocens Light, a termite of the Philippine Islands, under the name of P. multinucleata. In C. dudleyi Banks, a termite which occurs in the Canal Zone and which, like Pl. nocens, lives in dry wood and damages furniture and woodwork, the writer found a multinucleate flagellate very similar to it, yet sufficiently distinct to be assigned to a new species. In recognition of the work of Dr. C. A. Kofoid on this genus, it may appropriately be named P. kofoidi. The two flagellates correspond closely in most characteristics except the number and size of flagella and the arrangement of blepharoplasts in each unit. While in the type species there are described three very short flagella associated with each nucleus, each mastigont[1] of P. kofoidi possesses four long flagella.

1. Morphology and Behaviour.

A characteristic structure of Proboscidiella, as well as of Oxymonas, is the organelle of attachment called by Kofoid and Swezy a proboscis, but perhaps more appropriately termed a rostellum,[2] which, because of its great variability in length, is believed by them to be highly contractile. In the hind-gut of the termite flagellates may be closely crowded together, attached to the wall by these organelles (fig. 10, Pl. 22). Since in C. dudleyi, as in other termites, there is a chitinous intima lining this part of the intestine, the expanded ends of the rostella are not applied directly to the epithelial cells.

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[1] The term karyomastigont was used by Janicki for the complex in Stephanonympha and Calonympha consisting of one nucleus with the surrounding portion of the cytoplasm, the blepharoplast, the parabasal apparatus, the axial filament, and the flagella emerging from that blepharoplast. It is synonymous with the single neuromotor system with its associated nucleus, as used by Kofoid, except that this does not include the portion of cytoplasm. When the nucleus is lacking (in Calonympha) the term akaryomastigont is applied to the remaining structures, while mastigont may be used without reference to presence or absence of the nucleus, thus including both of the other terms.

[2] As used in metazoan morphology the term proboscis is applicable to

The intestinal faunas of many termites of the host species brought to New Haven and examined while living were found to consist of several species of flagellates, including Stephanonympha, Devescovina, a five-flagellated trichomonad and smaller forms, in addition to forms of Proboscidiella both with and without rostella. In shape and size the latter forms of Proboscidiella, although very variable, resembled Stephanonympha (36–96 × 31–78 microns). The flagella, which exceeded in length those of Stephanonympha, were all directed backward over the body and were in active motion, slowly propelling the organism forward. The area anterior to the nuclei, where, in other individuals, an organelle of attachment may be developed, was evenly rounded and clear. In the cytoplasm of these motile individuals were numerous spherical inclusions and particles of wood, the latter sometimes so large that the bodies were distorted to accommodate them. Rostellate forms were also abundant, though less so in this material than the motile forms. Although actively moving flagella were present on some of the rostellate individuals, others lacked those structures. The latter observation agrees to some extent with that made by Grassi on Microrhopalodina, that the attached individuals lacked flagella.

The body is usually broadly ovoidal or pyriform, narrowing

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an organ, usually tubular, developed in connexion with the head if not the mouth. The numerous structures grouped under this name are of diverse natures, many functioning in relation with feeding, while others have different uses, but not that of attachment. It is somewhat misleading to use this term for a structure of a unicellular organism which is not tubular nor associated in any way with feeding, but functions for attachment. These implications are avoided by use of the term rostellum, which, besides its use in botany, is applied to median, imperforate, more or less retractile organs of attachment on the scolices of cestodes.

towards the anterior end, from which the rostellum may be prolonged (figs. 14, 16 *a*, 18, Pl. 22). In a hundred individuals preserved on slides the length, exclusive of the rostellum, ranged from 23 to 165, averaging 66 microns, and the width at the broadest part of the body from 12 to 100, averaging 46 microns. Thus the length of P. kofoidi is similar to that of those individuals of P. multinucleata measured by Kofoid and Swezy, which ranged from 25 to 160 and averaged (in sixteen cases) 72 microns. On slides made from another colony (T–234) the average size was smaller, 50 × 38 microns.

On flagellates attached to the intestinal wall by the ends of the rostella, those organelles are generally relatively short and broad, though some are longer than in the individuals selected for illustration (fig. 10, Pl. 22). The end is broadly expanded and often is concave. On smears, however, the rostella are commonly longer, ranging up to 299 microns, which in this case was nearly four times the length of the body, 77 microns. Great lengths are infrequent, the usual size being from about 10 to 120, with an average in a hundred cases of 66 microns. While often the ends of the rostella are enlarged (fig. 16 *a*, 18, Pl. 22), frequently they are pointed or truncate (fig. 11, Pl. 22). The organelles may become broken off or detached from the flagellates, as evidenced by the fact that many isolated rostella were found on smears. It is possible, then, that some of those forms lacking rostella have not retracted the process, but it has been detached. In the rostellum there are a great many fibres, prolonged from the fibrillar system of the body (fig. 24, Pl. 23).

While oxymonads described or observed in twenty-eight species of Kalotermitinae are uninucleate, with occasional binucleate and rarely multinucleate stages, the two members of the genus Proboscidiella are predominantly multinucleate. In P. multinucleata uninucleate stages have been described, but the writer has found none in P. kofoidi. The smallest number of nuclei in several thousand individuals of the latter species was two, the largest number twenty-six (fig. 33, Pl. 24). In the latter instance the nuclei had separated into two groups of seven and nineteen, and binary fission had been proceeding.

Numbers above eleven are, however, infrequent. Though every count from two to nineteen, seldom more, occurs, eight is the most common number. This is borne out by records presented in the accompanying table from three hundred individuals on twelve slides. Of these sixty-five, which is by far the highest single number, had eight nuclei; the median number is eight and the average number in the whole group, disregarding the two exceptional cases of twenty-two and twenty-six, is 8·04.

FREQUENCY IN NUMBER OF NUCLEI IN 300 INDIVIDUALS.

2 – 4
3 – 7
4 – 18
5 – 24
6 – 42
7 – 39
8 – 65
9 – 27
10 – 20
11 – 19
12 – 7
13 – 6
14 – 5
15 – 1
16 – 7
17 – 3
18 – 2
19 – 2
22 – 1
26 – 1

On smears fixed in the Canal Zone soon after the termites of colony T–234 were secured, the bodies of Proboscidiella were almost without exception crowded to the base of the rostellum with yellow-brown spherules, some of which occurred among the blepharoplasts and fibrils anterior to the nuclei (fig. 16 *b*, Pl. 22). These spherules measured from 1 to 5, most commonly between 3 and 4 microns in diameter, and each was enclosed in a clear area, apparently a vacuole. While most of them were quite spherical in form, many were somewhat irregular in outline (fig. 17, Pl. 22). Sometimes these latter were irregularly ovoidal, in some there was a flattening or even a depression at one side, and in a few cases the globules were as irregular in outline as starch grains. The interior was not uniform in appearance, but seemed to be filled with a few large or many small vacuoles, and sometimes to contain also a few granules. When the body is ruptured these globules may remain intact after release.

In termites of another colony (T–239) which were brought to New Haven and fed for several days on filter paper, the cytoplasmic inclusions of Proboscidiella were not the same (fig. 11, Pl. 22; fig. 25, Pl. 23). There were fewer and smaller spherules, which did not have the same yellow-brown colour. They were usually not stained, but in material fixed in Flemming's fluid there were among them some, similar in outline to the others, which stained deeply with iron haematoxylin, as well as some with a deeply staining shell and inner granules (fig. 20, Pl. 23). Without doubt the filter-paper feeding had altered the cytoplasmic inclusions, so that the normal situation is not presented by flagellates from hosts which had been thus treated.

Spherules similar to those of this species of Proboscidiella are abundant in O. granulosa and occasionally, at least, occur in P. multinucleata, and in some species of Calonympha and Stephanonympha. In O. pediculosa Kofoid and Swezy found in many individuals large, spherical granules which stained deeply. Janicki suggests that in O. granulosa the spherules may be derived directly from the particles of wood, which acquire a rounded form. The facts which are known all point to the conclusion that they are derived from the ingested wood, perhaps as an extracted substance. Their lack of uniformity in internal structure makes it unlikely that they are fluid reserve products.

Among the spherules, in flagellates from normally fed termites, are small wood particles, the cytoplasmic fibres, often large fragments of wood, and tubular structures of variable thickness and length within which spherules may be enclosed. In a specimen the hosts of which had been fed upon filter paper for six days, there was, at the end of the bundle of axostyles (fig. 15, Pl. 22) a curious tubular structure of different nature. The tube was stained with iron haematoxylin, and thus is comparable with similar but shorter tubes in some other specimens (fig. 11, Pl. 22). The writer has been unable to form an opinion as to the nature of these structures. They are similar to certain cytoplasmic inclusions in P. multinucleata, which Kofoid has suggested may be 'vestigial sleeves' handed down from an Oxymonas-like ancestor.

A narrow zone of ectoplasm is distinct in some (fig. 11, Pl. 22) but not in all specimens. This seems to be alveolar in nature, as many fine lines, which are probably alveolar walls, traverse it. Inwardly the ectoplasmic layer, in which no cytoplasmic inclusions occur, is distinct from the endoplasm, and outwardly it is bounded by a thin pellicle.

Flagellates have been found in which there is a broad layer of granular protoplasm surrounding the body and, in some cases, partly covering the rostellum. When this substance covers an individual which lacks a rostellum, there is some resemblance to a cyst. The structure of this zone, which varies in width, is, however, not that of a cyst wall, but rather that of disintegrating protoplasm. A similar layer separating the pellicle from the wood-containing cytoplasm occurs in some individuals of Devescovina, Stephanonympha, and Calonympha, which are probably degenerate. In the intestine of the host, some moribund flagellates are most likely normally present, and the manipulations of smear-making certainly produce degenerative changes in some specimens. Consequently, although several specimens similar to that drawn by Kofoid and Swezy as a cyst of P. multinucleata have been found, these do not seem to the writer to afford convincing evidence for the occurrence of encystment in P. kofoidi.

A striking difference between the two species of Proboscidiella concerns the micro-organisms adherent to the surface of the body. In P. multinucleata the authors state (p. 311) that ' the surface of the body is generally covered with a dense coat of vertically attached bacteria ', and their figure shows rather long, rod-like forms. Possibly these are spiral organisms similar to those of certain species of Oxymonas, as these often appear like rods in fixed material. On the body of P. kofoidi there are instead short, slightly curved, rod-like micro-organisms. In a few cases these are absent, in others there are a few limited chiefly to the posterior region, while in many cases they are abundant on both the body and rostellum (fig. 19, Pl. 22). When sufficiently destained, the interior of these rods is clear, except for several large chromatic granules. The rods vary in length, and some are in stages of transverse fission. The micro-organism, which is also present in the gut contents, has not been observed in the living condition, but it is similar to a form, abundant in K. hubbardi, which moves with rapidity. The surface of Joenia annectens is generally covered with similar organisms in about the same numbers as those on P. kofoidi.

The nucleus is spherical or more frequently broadly ovoidal with the longer axis parallel to the longitudinal axis of the cell. Within the delicate membrane the nuclear material is arranged in a manner similar to that of Oxymonas (figs. 12, 13, Pl. 22). Most of the space is occupied by granules of chromatin somewhat unequal in size and arranged in groups or strands. There seldom, if ever, is a regular distribution of isolated granules on a reticulum. In the posterior region of the nucleus is a clear space in which is the karyosome surrounded, frequently, by a number of radial strands. The karyosome is somewhat irregular in outline; often it is broadly ovoidal with the longer axis transverse to the longer axis of the nucleus. The periphery is often more deeply stained, like a shell, and in the interior may be clear spaces. Sometimes there seems to be a central granule (figs. 34 *a*, 35, Pl. 24), such as that shown in P. multinucleata, but it is by no means certain that the structure really exists. At any rate there is so far no convincing evidence that a centriole exists within the karyosome. Besides the karyosome there may sometimes, but not often, be one or more similar masses in the nucleus.

The nucleus measures from 4 to 9, usually 6 to 7, microns in diameter, thus being of about the same size as that of the type species. The karyosome, however, is proportionately larger than in that flagellate. While, according to the text (Kofoid and Swezy, 1926, p. 304) the diameter of the karyosome in their species is not greater than one-fifth of the diameter of the nucleus, and is often smaller, that of this form usually measures fully a third and ranges from a quarter to a half of the nuclear diameter. On a set of smears prepared from T–234 the nuclei were smaller, 4 to 5 microns, and the karyosome diameter 0·2 to 0·25 of this. While less than that in the other race, this is still greater than that of P. multinucleata.

The fibrillar, or neuromotor, system consists of the same elements described by Kofoid and Swezy, but the arrangement of some of these is different. The numerous fine cytoplasmic fibres (figs. 11, 18, Pl. 22), termed by Kofoid retractor fibres, are not limited to the peripheral cytoplasm, as in P. multinucleata, but extend longitudinally throughout the endoplasm. Apparently they begin in the rostellum, where they constitute some of the rostellar filaments, run past the nucleus, and approach close to the posterior end of the body. Sometimes several of these cytoplasmic fibres are collected into a bundle.

Peripheral to the cytoplasmic fibres are the axostyles (fig. 20, &c., Pl. 23), which usually equal the nuclei in number, though often there are several more. Excepting in small individuals, in which the rostellum is short or absent, the axostyles do not reach the posterior end of the body, and may stop far short of this. Each axostyle is flattened, lies just under or in the ectoplasm, and is often turned outward at the posterior end to approach the pellicle, through which the tip may project. There is often some variation in the proportional length and thickness of axostyles in the same individual, and there may be considerable variation in this respect between those of different individuals (cf. figs. 11, 18, Pl. 22). After bending around the upper side of the nucleus, the axostyle tapers abruptly close beneath one of the blepharoplasts and gives origin to several fibres which continue into the rostellum (figs. 28, 29, Pl. 23). The axostyle seems to be composed of fibres, for some are split into several or divided completely up to the region of the blepharoplasts (figs. 22, 23, Pl. 23). In some cases the posterior end has a structure like that of the axostyle of Oxymonas, an arrow-shaped enlargement with a ring around the broader portion, and the part adjacent to this may be divided into several fibres (fig. 14, Pl. 22). It seems probable that the posterior portion, just described, has been lost in most instances, leaving only the tapering, more deeply staining portion (cf. p. 374).

It is by no means certain that the axostyle-like structures in Proboscidiella and Oxymonas are of the same nature as the axostyles of Trichomonas and Devescovina. They rather resemble the 'axostyles' of Pyrsonympha and Dinenympha, and perhaps the 'chromatic basal rod' of Devescovina. Like these, they stain deeply with iron alum haematoxylin, and not with Delafield's stain; like that of Pyrosonympha they may break up into fibres.

In the arrangement of blepharoplasts and flagella P. kofoidi differs from the type species. In the latter, according to the authors, 'the centrosome lies directly on the anterior face of the nuclear membrane. From it there passes anteriorly a short, delicate rhizoplast to the blepharoplast bar, from whose proximal end, constituting the primary blepharoplast, arises the single primary flagellum, while from the distal end, the secondary blepharoplast, emerges the pair of equal, secondary flagella'. The length of the flagella is shown as not more than three times the diameter of the nucleus, which is about six microns.

In P. kofoidi there are, in each mastigont, arising in pairs from the two blepharoplasts, four flagella of a length (about 60 microns) often equal to or exceeding that of the body (fig. 11, Pl. 22; figs. 20, 25, 27, Pl. 23). In this number and relative length of flagella this oxymonad agrees with several species of uninucleate oxymonads which the writer has observed (p. 359). Thus each single mastigont of the multinucleate form agrees in this particular with the uninucleate form, as is to be expected from the probable close evolutionary relationship between these flagellates.

In each mastigont the two blepharoplasts, which are some distance apart, are unequal in size and shape (fig. 27, Pl. 23). The larger primary blepharoplast, which is close to the anterior end of the nucleus and lies over the axostyle at the point where this narrows abruptly (figs. 28, 29, Pl. 23), is elongated and more pointed at the end opposite to that from which the two flagella arise. From this pointed anterior end there extends into the rostellum a fibre, the proximal section of which, when stout and deeply stained as it frequently is, appears as an oblique bar (fig. 27, Pl. 23). Situated more anteriorly and to the left of the primary blepharoplast is the more spherical secondary blepharoplast, from which also a fibre extends toward the rostellum. In most cases this fibre seems to meet that from the other granule, so that the two blepharoplasts seem to be at the ends of a fork unequal in length and thickness (figs. 27–9, Pl. 23), but since the fibres often run parallel as far as they can be traced, it is probable that they are not actually united. From the posterior end of each blepharoplast, at the outer edges as seen from above, two flagella emerge (figs. 27–9, Pl. 23). From the smaller secondary blepharoplast a filament runs posteriorly; sometimes this seems to meet the membrane of the adjacent nucleus (figs. 30–2, Pl. 23); sometimes it apparently ends freely (fig. 26, Pl. 23).

The fine interconnexions between axostyle, blepharoplasts, and nucleus are much more difficult to determine than are those structures described in the preceding paragraph, so that, although it is possible for the writer to describe them from a few observations, the description may not be entirely accurate. Under the primary blepharoplast the axostyle seems to end in a small enlargement which is connected to the blepharoplast by a very short strand (fig. 26, Pl. 23). Between this blepharoplast and the nucleus there is possibly a rhizoplast (fig. 26, Pl. 23), which seems to end in a granule at the point where it meets the membrane. In contrast to the stout blepharoplast bar described in P. multinucleata is the delicate strand which may sometimes be made out connecting together the two blepharoplasts (fig. 26, Pl. 23).

A brief summary of the above account will indicate how the blepharoplasts, axostyle, and nucleus of each mastigont are connected. The primary blepharoplast is perhaps attached by a rhizoplast to a granule (centrosome ?) on the nuclear membrane, to the axostyle by a short strand, and to the secondary blepharoplast by a fine filament. This blepharoplast also gives rise to a stouter filament which passes into the rostellum. From the secondary blepharoplast a similar filament also enters the rostellum, and another passes posteriorly to meet the membrane of the nucleus of the mastigont to the left, or to end freely between this and its own nucleus. To the membrane of that nucleus it may adhere, at some distance from the anterior end, in a manner similar to that in which the rhizoplast bands of

Staurojoenina are attached. Thus, by this and the primary rhizoplast, may the nuclei be suspended in position, if this account is correct.

No parabasal bodies similar to those of many other polymastigote flagellates have been found in P. kofoidi, despite careful search in material treated by methods which clearly demonstrated those structures in Devescovina and Stephanonympha on the same slides. In the position which the parabasal body would, if present, occupy a group of granules has sometimes been seen (figs. 25, 31, Pl. 23; fig. 11, Pl. 22). These granules are often in one or two rows, or grouped in a triangular form close to the nucleus near the primary blepharoplast. Similar granules may exist anterior to the nuclei, or scattered about in the anterior part of the body (fig. 25, Pl. 23), but the frequent observation of a group of these in the position mentioned suggests that they constitute a definite structure. Although not quite the same as the parabasal bodies of many other flagellates, this structure is probably of similar nature. No true parabasal body has been seen, to the writer's knowledge, in any oxymonad flagellate.[1]

2. Fission and Mitosis.

Multiplication by fission of the body, preceded by separation of the nuclei into two groups, unaccompanied by mitosis, occurs in P. kofoidi as well as in P. multinucleata. The numbers of nuclei in the two groups are generally unequal. Only one instance of equal separation was observed, with six nuclei in each set. Of those with unequal groups, at one extreme was a case in which there were two and three, and at the other extreme one with nineteen and seven nuclei (fig. 33, Pl. 24). In the earlier stages the nuclei have formed two groups, the axostyles are deeper in the body than usual, and the proximal, but not the distal, end of the rostellum is split, or at least the rostellar fibres are separated. Later the rostellum splits to the tip, and finally the two processes and two groups of nuclei are at opposite ends of the body, preliminary to final separation. In one case three groups of nuclei were seen in one body, an instance, probably, of multiple fission.

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[1] Since writing this the author has observed in a species of Oxymonas from Kalotermes clevelandi, fixed in Flemming's fluid without acetic acid, a granular structure exactly comparable to that of P. kofoidi.

Although a good many instances of mitotic organization have been found, these were limited to a few stages. The earliest changes begin while the nuclei are in their usual position. The chromatin draws away from the membrane, the karyosome moves toward the centre, and the strands of chromatin granules become thicker and more closely packed (fig. 35, Pl. 24). These changes occur synchronously in all nuclei of an individual, and are most frequent on slides where there are other stages of mitotic organization. There are instances of degenerating nuclei in which the chromatin is similarly clumped, but these occur in only one or two nuclei of a group and are different in appearance from the prophase organization stages.

In later stages the rostellum is shorter and the nuclei have migrated from their usual position into the posterior cytoplasm (fig. 34, Pl. 24). Blepharoplasts, axostyles, cytoplasmic fibres and flagella remain in place. It has not been possible to determine whether or not a centrosome, lying upon the membrane, migrates with the nucleus.

In the migrating prophase nuclei (fig. 34, Pl. 24) the chromatin is dispersed and organized into groups and threads of chromatin granules, perhaps following a contraction stage such as that previously described. The karyosomes, which have moved toward the centre of the nuclei, are of the usual size and form, some showing an inner clear region in which, especially under low magnification, there sometimes seems to be a granule. More careful observation, however, renders the existence of this granule doubtful.

Mitosis proceeds in the nuclei free in the cytoplasm, as in Lophomonas, Calonympha, Stephanonympha, and Oxymonas (fig. 37, Pl. 24). Apparently the number of dividing nuclei at least frequently approximates eight. Thus, in six cases of karyokinesis, in which the nuclei had migrated from their usual position, there were 8, 8, 7, 7, 7, and 6 of these. In a number of others the bodies had been ruptured, the old fibrillar apparatus torn away, leaving in the remaining cytoplasm 10, 7, 7, and 4 nuclei.

The mitotic figures are very different from those of any other flagellates of termites which have been described, except Oxymonas. In the earliest spindle stage observed (fig. 36, Pl. 24), the nuclear membrane, which is more distinct than in the resting stage, is only slightly elongated (about 8 × 7 microns). Within the membrane the most conspicuous structure is the curved centrodesmose, which is about 1·5 microns broad. Where the truncate ends of this touch the membrane, that is indented as if drawn inward. Surrounding the centrodesmose is chromatic material in the form of numerous short, varicose strands or linear groups of granules.

Later the nucleus becomes more elongated and its ends more pointed. No longer is the membrane drawn inward at the ends of the centrodesmose, but rather it is pushed outward. The centrodesmose, which, when sufficiently destained, seems to consist of a stainable shell with a clear core, has become more bowed (figs. 39, 42, Pl. 24), as if its increase in length has been greater than that of the nucleus. The convex margin may appear stouter and stain more deeply, as if there were a coarse, stainable strand there (fig. 42, Pl. 24), but that is not always the case. In some cases there is an indication of a central strand (fig. 40, Pl. 24), but this may be an illusion due to uneven staining of the shell.

Surrounding the centrodesmose is the spindle, with fibres converging to the poles. While the bulk of the spindle is on the concave side of the centrodesmose, it also extends around the convex side (fig. 39, Pl. 24). Apparently there are no definite chromosomes; at least, if there are, these have no regularity of form and are very numerous. In the mass of chromatic material there are some single granules, but more frequently these granules are collected into linear groups (figs. 37*a*–42, Pl. 24). The chromatin belt may extend continuously from pole to pole, may be more restricted to the equatorial region, or may be separated into two belts as in an anaphase.

In many instances, and possibly in all, there is a mass on the spindle comparable in size to the karyosome (figs. 41, 42, Pl. 24). This mass is often irregular in form, or may be spherical and much like the karyosome of the resting nucleus in appearance, even to the apparent inner granule. In some later spindles this body seems to be constricting in a dumb-bell form.

At about this time there has begun to differentiate what is most likely the new axostyle (figs. 40, 43, Pl. 24). This is a deeply staining strand, tapering to a point at each end and in length about half that of the nucleus. One pointed end is close to the end of the centrodesmose, from which it is separated by the nuclear membrane and a small space in the cytoplasm. No granule nor rhizoplast has been detected at the end of this structure, even in material prepared in such a manner as to demonstrate clearly the blepharoplasts.

A flagellate without a rostellum (fig. 14, Pl. 22), with nuclei and axostyles different in structure from those of most other specimens, may be interpreted as in a stage following mitotic division. The axostyles differ from the usual condition in each having a posterior prolongation consisting of several filaments which finally collect into an arrow-shaped enlargement. Besides the karyosome, many of the nuclei contain one or two other masses similar in size, form, and stainability. It is possible that the posterior prolongation of the axostyle is later lost, and the nucleus takes on the more typical resting appearance. In the cytoplasm of this flagellate are several structures which seem to be degenerating nuclei, as if all the products of mitosis had not survived.

While this series of stages is too incomplete for the solution of several problems of fundamental importance, it makes possible an incomplete interpretation of the type of mitosis. In P. kofoidi nuclear division proceeds in the same general manner as in Oxymonas, in which, however, there are generally more definite chromatin strands. Although no direct evidence as to the origin of the centrodesmose has been obtained, it is probable that this develops from the karyosome, as in Euglenoidina and, according to Janicki, in O. granulosa.

The origin of the karyosome-like body seen on the spindle is obscure. At the time of mitosis the blepharoplasts, flagella, axostyles, and fibres are discarded, and probably new organelles are differentiated.

The existence of an extranuclear division centre in the oxymonad flagellates, which is to be expected, awaits conclusive demonstration. None has been recorded in Oxymonas nor in Proboscidiella, excepting that Kofoid and Swezy found a granule which they refer to as a centrosome on the nuclear membrane of P. multinucleata and two such granules on the nucleus of O. projector. They were unable, however, to follow the fate of these granules during nuclear division. It has not been possible to detect any centrosomes nor paradesmose in mitotic figures of P. kofoidi, but the material has been limited. Mr. C. C. Zeliff has obtained evidence of the existence of centrosomes and a delicate paradesmose in a uninucleate oxymonad from Rugitermes kirbyi.

3. Racial Differences.

Most of the observations recorded in this paper were made on P. kofoidi from termites of colony T–239, but upon comparison with those from T–234 several differences, most of which have already been mentioned, were detected. On slides prepared in the Canal Zone from the latter colony, the flagellates were smaller, the organelles of attachment longer, the average number of nuclei was less, the nuclei were smaller, the spherical cytoplasmic inclusions were different in number and size, and the body-surface was free of the micro-organisms so characteristic of the others (fig. 16, Pl. 22). While in the flagellates from T–239 the average size was 66 × 46 microns, and forms exceeding 100 microns in length of the body proper were not uncommon, in those from the other colony the average was only 50 × 38 microns, and no individuals of exceptionally large size were observed. Long rostella were more common than in the others, and the average length of twenty of these was 50 per cent. greater. In one case the rostellum was nearly five times the length of the body (220 and 45 microns). In a hundred individuals the number of nuclei ranged from 2 to 11, averaging 5·97 and with 6 as the median number. Besides being less numerous, the nuclei were smaller in size, 4 × 5 microns, and the karyosomes measured only 0·2 to 0·25 of this. The cytoplasm of these flagellates was, as already mentioned, crowded with large spherules (p. 364), while in the Proboscidiella examined from termites of colony T–239 these were smaller and less numerous, although under normal conditions they may have been the same. The presence of micro-organisms on flagellates from the termites brought to New Haven was a constant characteristic, but these were absent from the other material.

It is, of course, possible that transportation and filter-paper feeding produced changes in the intestinal flagellates, but it seems unlikely that those factors would modify any of the characteristics referred to except the spherules in the cytoplasm. In comparison with what may occur in other flagellates of termites treated in a similar manner, it is probable that the unusual conditions would, if anything, decrease the size and stimulate division, besides altering the cytoplasmic contents.

These differences in the flagellates from the two host colonies may represent racial peculiarities which have developed during a long period of isolation, or may be within the normal range of variation. At any rate, this demonstration of the differences which may occur shows the need of caution in the classification of similar flagellates, for, if the hosts had been of two species, specific separation of the two forms of Proboscidiella would have seemed justifiable. As it is, it seems best to regard the smaller flagellate as a variety of the species kofoidi, of which the type is the form from colony T–239.

4. Systematic Position of Species.

The differences between the flagellar apparatus of those species of uninucleate oxymonads mentioned in the first part of this paper, and those species of Oxymonas described by Kofoid and Swezy (1926), lead to some confusion as to the characters of the group. The former agree in number and relative length of flagella with each mastigont in P. kofoidi, while the latter have twice the number possessed by each neuromotor unit of P. multinucleata. A constant difference in the number of flagella is justification for the separation of genera in many flagellates. No doubt this should also be the case with the oxymonads, unless further study shows that there is greater uniformity than present descriptions seem to indicate. Although the writer was inclined at first to assign the Central American species of multinucleate oxymonad to a new genus, further consideration of the close resemblance in most respects between it and the described species of Proboscidiella made it seem more likely that it should rather be placed in that genus. If that is the correct procedure, it is at least necessary to modify the characters of the genus as regards number of flagella to permit the inclusion of this species with four flagella in each mastigont.

5. Diagnosis: Proboscidiella kofoidi sp. nov.

Entozoic flagellates with the characters of the genus, but differing from the generic type in having no blepharoplast bar but two blepharoplasts possibly connected by a delicate filament, and two pair of long flagella in each mastigont. The number of mastigonts varies from two to nineteen or more, averaging eight. The body normally contains numerous characteristic spherules, as well as particles of wood. The flagellates are generally attached to the lining of the gut by an anterior prolongation of the body, but may lack that structure and swim in the lumen by means of the long flagella. The average size is about 66 × 46 microns, and the average length of the rostellum about that of the body. Short, rod-shaped micro-organisms are often attached in large numbers to the surface of the body, by one end.

Host: Kalotermes (Cryptotermes) dudleyi Banks, 1918. Colony T–239. Locality: Balboa, Canal Zone, Panama.

Variety in Kalotermes (Cryptotermes) dudleyi. Colony T–234. Locality: Ancon, Canal Zone, Panama.

(For characteristics see p. 375.)

V. THE GENUS MICRORHOPALODINA AND GRASSI'S FLASK-SHAPED FORMS OF CALONYMPHIDAE.

The account of Microrhopalodina enflata given by Grassi and Foa (1911) is very incomplete and lacks illustrations, but it is probable that they were describing a multinucleate oxymonad. This flagellate, according to them, has a brief period of free existence, and a much longer period of attachment to the intestinal lining by a long 'peduncle'. In the fixed stage the flagella are lost, growth occurs, a flask-form is assumed, and the nuclei, blepharoplasts, and axostyles multiply repeatedly, forming a group at the base of the 'neck'. From near the nuclei the axial filaments may continue anteriorly to the attached extremity of the neck, and many may run posteriorly into the body. In this stage solid wood is ingested and there are in the cytoplasm numerous round corpuscles which, though not fat, are possibly some reserve material. When the termite moults, the flagellates become detached, the neck disappears, the characteristic corpuscles and wood are discarded, and flagella develop. This form may then divide into small, uninucleate flagellates, which give origin to new attached flask-forms.

From this description, since we receive no information concerning size, number, or structure of nuclei, number or length of flagella, arrangement of blepharoplasts or size of the body, it is impossible to gain a clear conception of Microrhopalodina. Of those flagellates in termites which are known, Proboscidiella agrees most closely with the description, in that the nuclei are located in both at the base of the 'neck', for the most part, rostellar and body-filaments are present, the anterior organ of attachment may be well developed or lost, flagella may develop, especially on free forms, and characteristic corpuscles as well as wood particles may be present in the body. Perhaps a re-study of Microrhopalodina will show that it has the same structure as Proboscidiella, in which case the latter name will become a synonym of the former, which will be the type genus of the family.[1]

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[1] Through the kindness of Dr. A. E. Emerson, of the University of Pittsburg, the writer has been able to examine two living nymphs of

The uninucleate, rostellate flagellates figured by Grassi in 1917 were regarded by him as stages in the life-history of Calonymphidae, to which family he also assigns Microrhopalodina. The so-called flask-forms of S. silvestrii var. neotermitis erythraei resemble Oxymonas, while the larger flask-forms attributed to Diplonympha foae, which have more highly developed rostella but only one or sometimes two nuclei, are not unlike O. pediculosa and the form from K. panamae. If any more than morphological evidence is needed to show the error, which Bernstein (1928) has pointed out, in Grassi's interpretation of these flagellates, this is supplied by the fact that in K. minor and K. hubbardi, which harbour uninucleate oxymonads, no Calonymphidae nor other multinucleate flagellates are present.[1]

The family Calonymphidae, in which Grassi placed the above-mentioned Protozoa, is defined by him as comprising flagellates with many nuclei, many axial filaments, blepharoplasts, and parabasal bodies. While Proboscidiella would be covered by that definition, except for the lack of definite parabasal bodies, Oxymonas would not, and obviously Oxymonas cannot be separated from Proboscidiella. Because of this fact, together with the marked morphological differences, the oxymonad flagellates should be removed from the family Calonymphidae, in which they have recently been included by Bernstein (1928).

VI. THE FAMILY OXYMONADIDAE.

The oxymonad flagellates, which form a unified group with characteristic structures and peculiar distribution, are set apart in several respects from all other flagellates known at present.

K. flavicollis. These were infected with an abundance of Joenia annectens, Hexamastix termitis, Janickiella grassii, and the so-called Trimitus-forms, but no specimens of Microrhopalodina were found.

[1] Grassè (1926, p. 572) states that he has found the uninucleate stages of M. enflata in K. flavicollis, and accepts Grassi's interpretation of similar flagellates as developmental stages of Stephanonympha.

It is therefore very desirable that a family be established to contain them, in order that they may no longer be confused with quite different forms. For convenience in classification the writer proposes the new family Oxymonadidae.

1. Diagnosis: Oxymonadidae fam. nov.

Entozoic flagellates which have an anterior organelle of attachment, the rostellum, possess a characteristic nucleus, and have a peculiar type of mitosis. The nucleus contains a large karyosome and granular chromatin filling the remaining space. During division, there is a stout centrodesmose, which is apparently developed from the karyosome. There are in each mastigont two blepharoplasts, each of which gives rise to a group of flagella (two or three in all cases except P. multinucleata), an axostyle, probably a parabasal (see p. 371), and cytoplasmic and rostellar filaments. The majority of known species are uninucleate, but there are also multinucleate forms of some of these species and multinucleate species. So far as is known at present these flagellates occur only in termites of the sub-family Kalotermitinae, in which they are widely distributed.

2. List of Genera and Species.

(?) Microrhopalodina Grassi and Foa, 1911.

M. enflata Grassi and Foa, 1911.

Host: Kalotermes (Kalotermes) flavicollis Fabr.
Locality: Italy.

(Inadequately described and not figured.)

Oxymonas (Janicki, 1915) emend. Kofoid and Swezy, 1926.

O. granulosa Janicki, 1915.

Host: Kalotermes (Neotermes) connexus Snyder, 1922.
Locality: Honolulu, Hawaii.

O. projector Kofoid and Swezy, 1926.

Host: Kalotermes (Glyptotermes) perparvus Emerson, 1925.
Locality: Kartabo, British Guiana.

O. pediculosa Kofoid and Swezy, 1926.
Host: Kalotermes (Lobitermes) nigriceps Emerson, 1925.
Locality: Kartabo, British Guiana.

O. gracilis Kofoid and Swezy, 1926.
Host: Kalotermes (Rugitermes) magninotus Emerson, 1925.
Locality: Kartabo, British Guiana.

O. parvula Kirby, 1926.
Host: Kalotermes (Cryptotermes) hermsi Kirby, 1925.
Locality: Fanning Island, Central Pacific Ocean.

Proboscidiella Kofoid and Swezy, 1926.

P. multinucleata Kofoid and Swezy, 1926.
Host: Kalotermes (Planocryptotermes) nocens Light, 1921.
Locality: Manila, Philippine Islands.

P. kofoidi Kirby, 1929.
Host: Kalotermes (Cryptotermes) dudleyi Banks, 1918.
Locality: Balboa, Canal Zone, Panama.

3. Systematic Position of Family.

Wenyon (1926) separates the monozoic, diplozoic, and polyzoic flagellates as Protomonadida, Diplomonadida, and Polymonadida. In this scheme the Oxymonadidae have no place, for the family includes both monozoic and polyzoic forms, and there would certainly be no justification for distributing Oxymonas and Proboscidiella into separate orders. For them it is better to retain the order Polymastigida, as does Calkins (1926), which includes the polyzoic Calonymphidae, besides other flagellates. Therefore, although in nuclear structure and mitotic division the Oxymonadidae differ from other Polymastigada, they may be assigned to that order, near the Dinenymphidae.

VII. Appendix: List of Kalotermitinae Examined for Protozoa.

Preparations from the following termites of the sub-family Kalotermitinae are available to the writer for the study of intestinal Protozoa.[1]

Kalotermes (Calcaritermes) brevicollis Banks, 1918. Barro Colorado Island, Canal Zone.

Kalotermes (Calc.) emarginicollis Snyder, 1926. Estrella, Costa Rica.

*Kalotermes (Cryptotermes) brevis Walker, 1853. Porto Rico.

Kalotermes (Crypt.) breviarticulatus Snyder, 1926. Taboga Island, Panama.

Kalotermes (Crypt.) dudleyi Banks, 1918. Balboa, Canal Zone.

Kalotermes (Crypt.) hermsi Kirby, 1925. Fanning Island.

Kalotermes (Glypt.) barbouri Snyder, 1924. Barro Colorado Island, Canal Zone.

Kalotermes (Kalotermes) clevelandi Snyder, 1926. Ancon, Canal Zone.

Kalotermes (K.) contracticornis Snyder, 1925. Cartago, Costa Rica.

*Kalotermes (K.) flavicollis Fabricius. Europe.

*Kalotermes (K.) hubbardi Banks, 1920. California.

Kalotermes (K.) immigrans Snyder, 1922. Fanning Island.

*Kalotermes (K.) jouteli Banks, 1920.

Kalotermes (K.) marginipennis Latreille, 1811. Cartago, Costa Rica.

Kalotermes (K.) minor Hagen, 1858. California.

Kalotermes (K.) panamae Snyder, 1924. Barro Colorado Island, Canal Zone.

*Kalotermes (K.) schwarzi Banks, 1920.

Kalotermes (K.) tabogae Snyder, 1924. Taboga Island, Panama.

Kalotermes (Lobitermes) longicollis Banks, 1918. Taboga Island, Panama.

Kalotermes (Neotermes) holmgreni Banks, 1918. Taboga Island, Panama.

Kalotermes (Rugitermes) kirbyi Snyder, 1926. Cartago, Costa Rica.

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[1] For the material from those species which are starred, I am indebted to Dr. A. E. Emerson, Dr. S. F. Light, Mr. G. F. Hill, and Dr. L. R. Cleveland. In preparation of this list I have been assisted by Dr. T. E. Snyder.

*Porotermes (Porotermes) grandis Holmgren, 1912. Victoria, Australia.
*Kalotermes (Cryptotermes) species. Gardner Island, Galapagos.
*Kalotermes (K.) species. Gardner Island, Galapagos.
Kalotermes species. Estrella, Costa Rica.

Oxymonad flagellates have been found in all of these excepting Porotermes grandis. In addition, oxymonad flagellates have been found in the following species, as recorded in the literature :

Kalotermes (Glypt.) perparvus Emerson, 1925. Kartabo, British Guiana.
Kalotermes (Glypt.) parvulus Sjöstedt. Africa, near Gold Coast.
Kalotermes (Neotermes) erytraeus Silvestri. Eritrea.
Kalotermes (Neo.) connexus Snyder, 1922. Honolulu, Hawaii.
Kalotermes (Lobitermes) nigriceps Emerson, 1925. Kartabo, British Guiana.
Kalotermes (Planocryptotermes) nocens Light, 1921. Manila, Philippine Islands.
Kalotermes (Rugitermes) magninotus Emerson, 1925. Kartabo, British Guiana.

The following species have been investigated for Protozoa but Oxymonadidae have not been recorded. They are not, however, known to be absent.

'Epicalotermes aethiopicus Silvestri.' Eritrea.
Kalotermes (Crypt.) havilandi Sjöstedt. Africa, near Nigeria.
Kalotermes (Glypt.) iridipennis Froggatt. Australia.
Porotermes (Porotermes) adamsoni Froggatt. Australia.

REFERENCES.

Bernstein, T. (1928).—" Untersuchungen an Flagellaten aus dem Darmkanal der Termiten aus Turkestan ", 'Arch. f. Prot.', vol. 61, pp. 9–37, pls. 1–2.

Calkins, G. N. (1926).—' The Biology of the Protozoa.' Philadelphia and New York : Lea and Febiger.

Grassè, P. P. (1926).—" Contribution à l'étude des flagellés parasites ", 'Arch. Zool. Exp. Gen.', vol. 65, pp. 345–602.

Grassi, B. (1917).—" Flagellati viventi nei Termiti ", 'Mem. R. Accad. Lincei ', (5), vol. 12, pp. 331–94, pls. 1–10.

Grassi, B., and Foa, A. (1911).—" Intorno ai protozoi dei termitidi ", 'Ren. R. Accad. Lincei ', (5), vol. 20, 1 sem., pp. 725–41.

Janicki, C. (1915).—" Untersuchungen an parasitischen Flagellaten.

II Teil: Die Gattungen Devescovina, Parajoenia, Stephanonympha, Calonympha ", 'Zeit. wiss. Zool.', vol. 112, pp. 573–691, pls. 13–18.

Kirby, H. (1926).—" The intestinal flagellates of the termite, Cryptotermes hermsi Kirby ", 'Univ. Calif. Publ. Zool.', vol. 29, pp. 103–20, pls. 8, 9.

Kofoid, C. A., and Swezy, O. (1926 *a*).—" On Oxymonas, a flagellate with an extensile and retractile proboscis from Kalotermes from British Guiana ", 'Univ. Calif. Publ. Zool.', pp. 285–300, pl. 30.

—— (1926 *b*).—" On Proboscidiella multinucleata gen. nov., sp. nov., from Planocryptotermes nocens from the Philippine Islands, a multinucleate flagellate with a remarkable organ of attachment ", ibid., vol. 28, pp. 301–16, pls. 31, 32.

Wenyon, C. M. (1920).—'Protozoology, a manual for medical men, veterinarians and zoologists.' New York: William Wood & Co.

DESCRIPTION OF PLATES 21–24.

All figures were drawn with a camera lucida at magnifications of 1,440 and 2,740, excepting figs. 10, 16 *a*, 18, and 25. The methods of fixation and staining used for each specimen are indicated in the description of the figure as follows: S., Schaudinn's fluid; F., Flemming's fluid without acetic acid; D., Delafield's haematoxylin; H., Heidenhain's iron haematoxylin.

PLATE 21.

Figs. 1–9.—Uninucleate oxymonad flagellates with four flagella. Drawings were made with the camera lucida from dark-field preparations of living material. All figures, except fig. 7, were drawn to the scale of microns. ×900.

Figs. 1–2.—From K. hubbardi. Oxymonads lacking rostella, with four flagella and attached spirochaetes.

Fig. 3.—From K. hubbardi. Spirochaetes removed by feeding host with acid fuchsin; some rod-shaped bacteria adherent to body.

Fig. 4.—From K. hubbardi. A small rostellate individual, with four flagella and adherent spirochaetes.

Fig. 5.—From K. minor. Large number of attached spirochaetes. Flagella not shown.

Fig. 6.—From K. minor. Outline drawing showing flagella.

Fig. 7.—From K. minor. Anterior end of a crushed but still living individual, showing two flagella from each blepharoplast, axostyle, and nucleus. ×1,712.

Fig. 8.—From K. marginipennis.

Fig. 9.—From K. tabogae.

PLATE 22.

Figs. 10–19.—Proboscidiella kofoidi sp. nov.

Magnification as stated. Figs. 10 and 19 drawn to adjacent scales; fig. 16 *a* to scale of fig. 19; figs. 11, 12, and 17 to scale at top; others to scale at lower left.

Fig. 10.—Section of intestine of Cryptotermes dudleyi. Flagellates attached by rostella to wall. × 490. S.D.

Fig. 11.—Full length of flagella; body filaments; granules in position of parabasal body and at base of rostellum. × 900. S.H.

Fig. 12.—Resting nucleus *a* is a median optical section; *b* a surface view. × 1,712. S.H.

Fig. 13.—Resting nucleus. × 1,712. S.H.

Fig. 14.—Individual without rostellum. Arrow-shaped enlargements at ends of axostyles, before which the axostyles are split into fibres. Several possibly degenerating nuclei in cytoplasm. × 900. F.H.

Fig. 15.—Rostellum absent, axostyles and filaments gathered into bundle. Tubular structure at end of bundle not connected to axostyles. × 900. S.H.

Fig. 16.—Variety from colony T–234. *a*, Very long rostellum, expanded at tip. × 375. S.D. *b*, Same organism, showing large spherical corpuscles crowding cytoplasm. × 900. S.D.

Fig. 17.—Spherical corpuscles similar to those of fig. 16 *b*. Note clear areas within and depressions on one side of some. × 1,712. S.D.

Fig. 18.—Large individual, long rostellum expanded at tip, only basal portion of flagella shown. Spherical corpuscles, fibrillar system, and filter-paper fibres in body. × 375. S.H.

Fig. 19.—Micro-organisms adherent to surface of body. These are present generally, but have been omitted from most figures. × 900. S.H.

PLATE 23.

Figs. 20–32.—Proboscidiella kofoidi sp. nov.

Figs. 20–3 and 25 were drawn to scale on left. × 900. All others to scale of microns on right. × 1,712.

Fig. 20.—Full length of flagella; characteristic corpuscles in cytoplasm, and some which stain deeply. S.H.

Fig. 21.—Three nuclei; characteristic form of rostellum. The flagella are omitted. S.H.

Figs. 22–3.—Splitting of the axostyles into fibres. Fig. 22 is focussed on the lower portion, fig. 23 on the upper of the same specimen. Blepharoplasts and rostellar filaments also shown. F.H.

Fig. 24.—End of a broad rostellum, showing rostellar filaments. S.H.

Fig. 25.—Diagrammatic typical figure, showing rostellum, flagella, axostyles, rostellar filaments, cytoplasmic filaments, corpuscles and ingested particles in body, and deeply stained granules in position of parabasal body and elsewhere.

Figs. 26–32.—Detailed diagrams showing arrangement of nuclei, blepharoplasts and associated filaments, and origin of flagella.

Fig. 26.—Rhizoplast, interblepharoplast filament, connexion between axostyle and blepharoplast, and filament from secondary blepharoplast to nucleus. F.H.

Fig. 27.—Group of nuclei and blepharoplasts, showing flagella and filaments from blepharoplast to rostellum, one of which is stout at base. Axostyles omitted. S.D.

Figs. 28–9.—Origin of flagella, axostyles with anterior prolongations, filaments from blepharoplasts into rostellum. F.H.

Fig. 30.—Filament from secondary blepharoplasts to nuclei. F.H.

Fig. 31.—Same, and collection of granules in position of parabasal body. F.H.

Fig. 32.—Filament from secondary blepharoplast to adjacent nucleus. F.H.

PLATE 24.

Figs. 33–43.—Proboscidiella kofoidi sp. nov. Fission and mitosis.

The drawings of entire organisms (figs. 33, 34, 37) were drawn to scale near top. × 800. Those of individual nuclei by scale in lower left. × 1,522.

Fig. 33.—Fission. The nuclei have separated into two groups of 19 and 7, and the rostellum has divided. S.H.

Fig. 34.—Nuclei in early prophase, migrating into posterior region of cytoplasm. S.H.

Fig. 34 *a*.—Nucleus from fig. 34.

Fig. 35.—Condensed chromatin mass. Probably earlier than fig. 34. Flagellates may be seen in which all the nuclei, in the usual position near the blepharoplasts, are in this condition. S.H.

Fig. 36.—Later prophase nuclei in portion of ruptured body. Note indentations in membrane at ends of centrodesmose. S.H.

Fig. 37.—Later prophase nuclei, migrated from anterior region. The blepharoplasts are in their usual position. S.H.

Fig. 37 *a*.—Single nucleus from fig. 37.

Fig. 38.—Hollow appearance of centrodesmose; spindle fibres and chromatin belt. S.H.

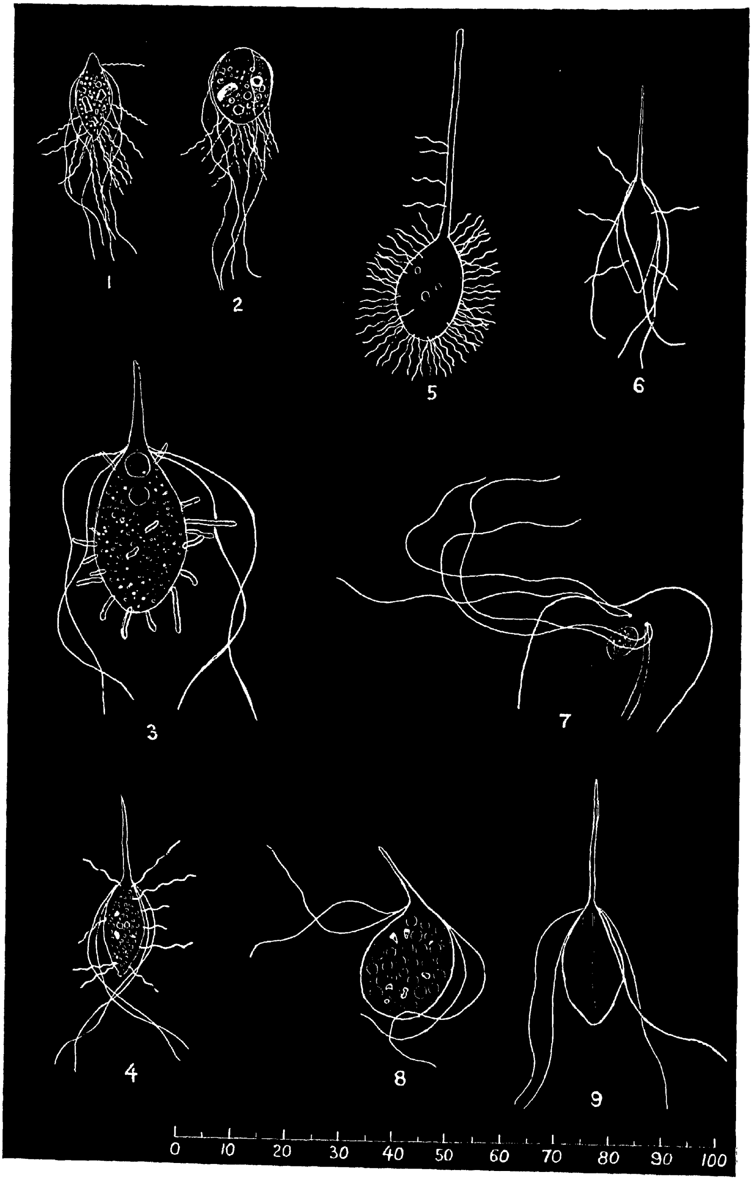
Fig. 39.—Increased curvature of centrodesmose. S.H.

Fig. 40.—Appearance of new axostyle (?) at one pole. S.H.

Fig. 41.—Chromatic mass on spindle. Aggregations of chromatin granules separating into two groups. S.H.

Fig. 42.—Similar. Hollow appearance of centrodesmose; denser stain on convex side; chromatic mass on spindle; groups of chromatin granules collecting toward poles. S.H.

Fig. 43.—Spindle-formed nuclei; development of new axostyles. S.H.



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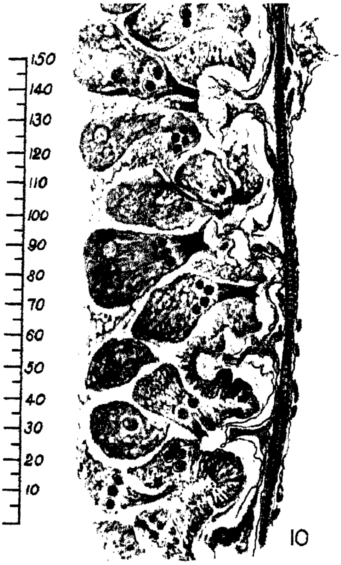

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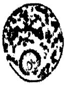

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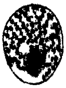

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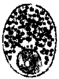

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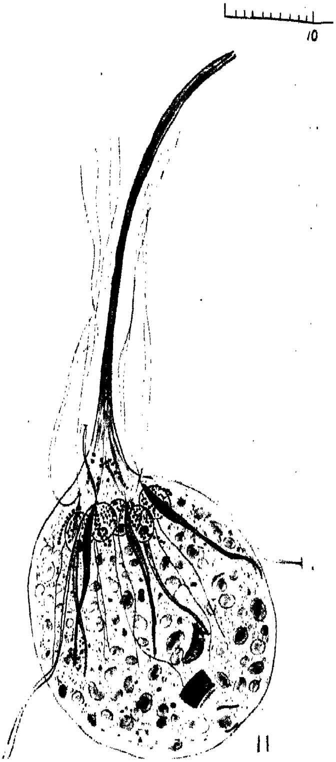

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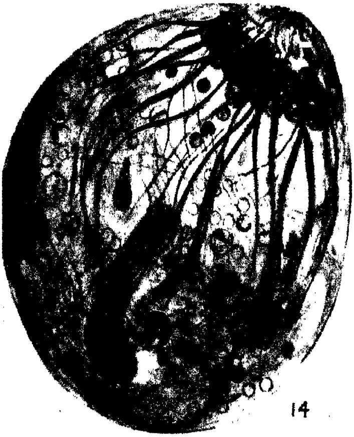

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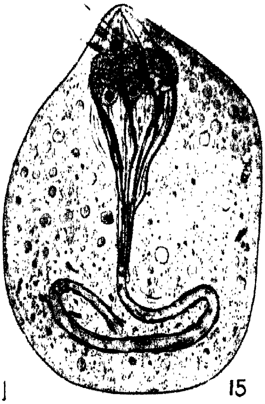

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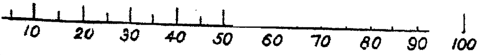

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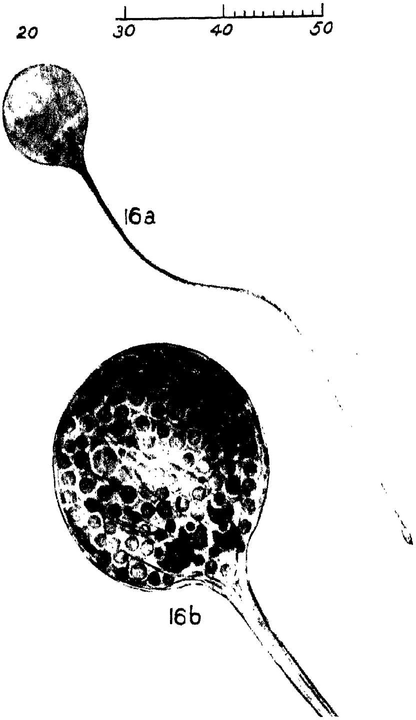

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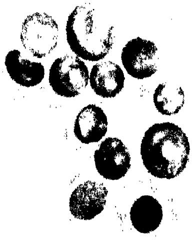

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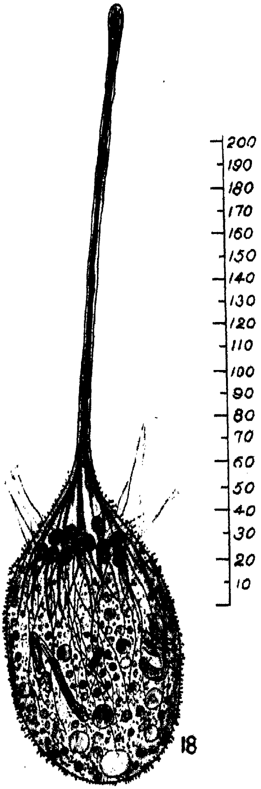

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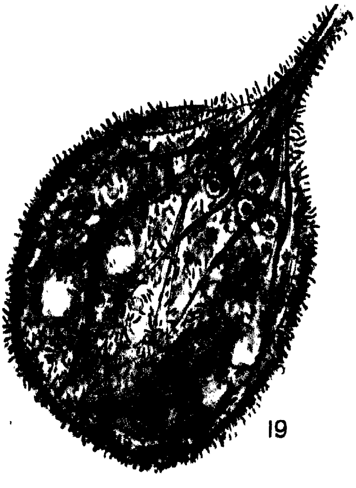

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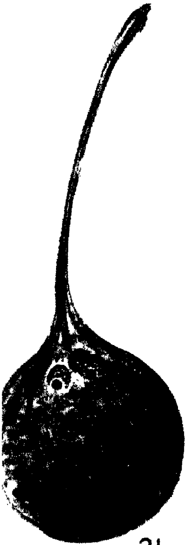

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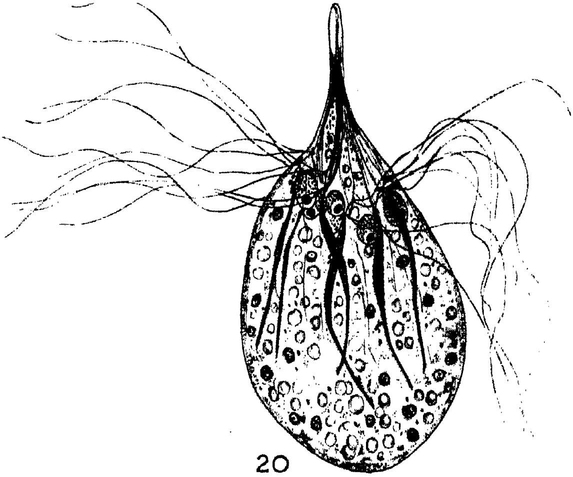

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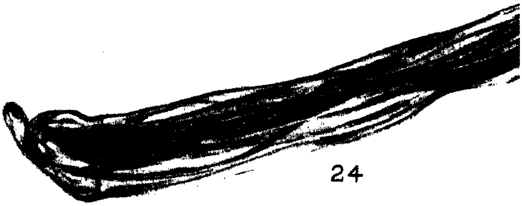

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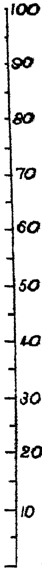

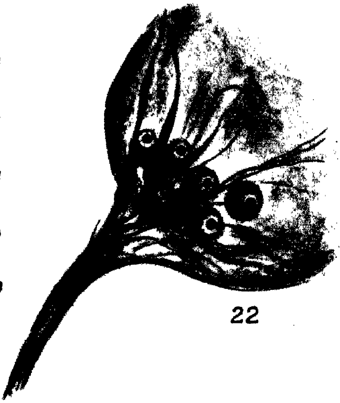

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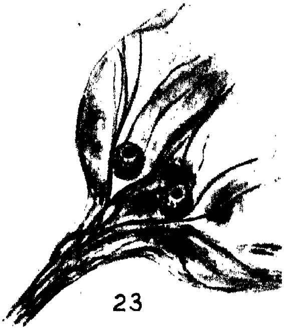

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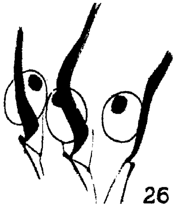

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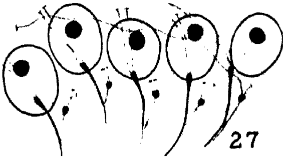

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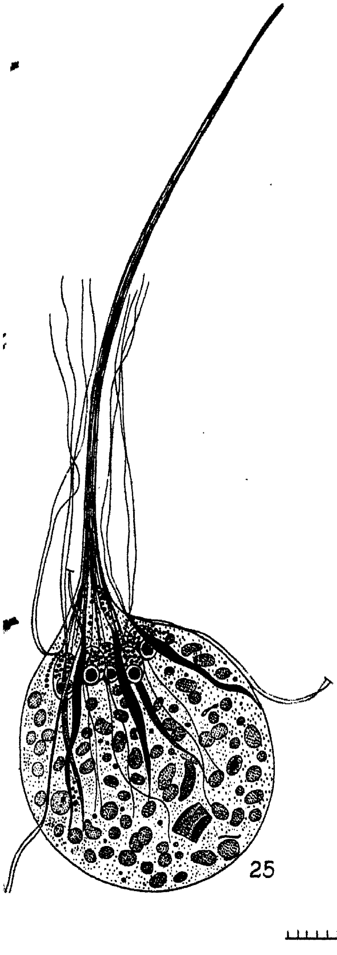

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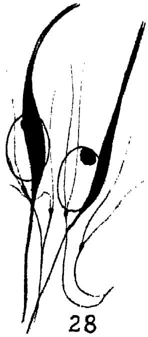

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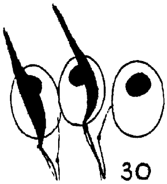

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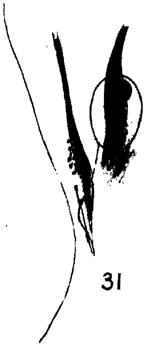

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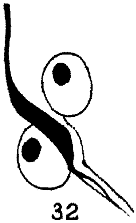

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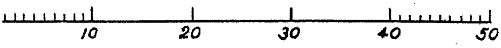

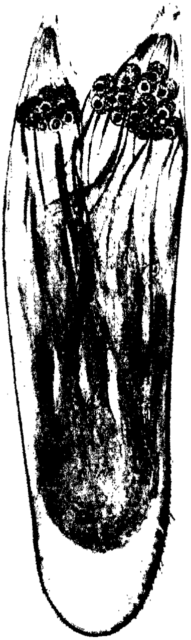

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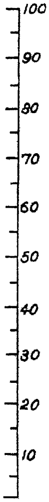

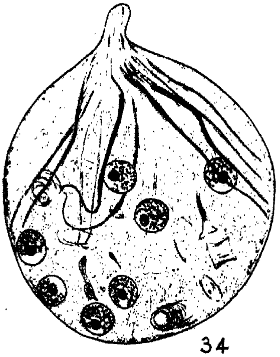

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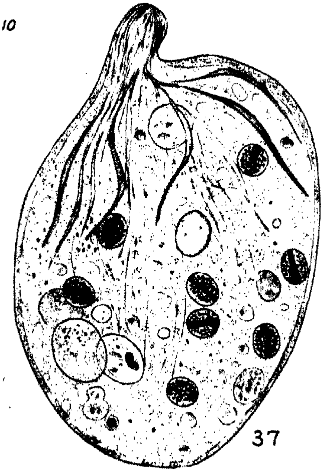

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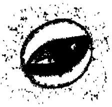

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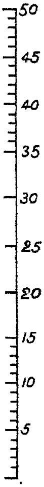

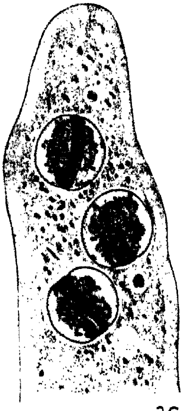

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H. Kirby and L. Krause, del.

The Cytoplasmic Inclusions of Certain Plant Cells.

By

Ruth Patten, Margaret Scott, and J. Brontë Gatenby.

From the Zoological Laboratory, Trinity College, Dublin.

With Plate 25 and 10 Text-figures.

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INTRODUCTION.

RECENTLY Bowen (1, 2) has announced the discovery of osmiophilic platelets in plant cells, and has suggested that these are the homologues of the Golgi bodies of animal cells.

This interpretation of the nature of the osmiophilic platelets is one which finds favour with the senior author for a number of reasons.[1]

---

[1] They consistently impregnate with two Golgi methods universally used by animal cytologists—the Kolatchev and Mann-Kopsch methods.

They are the same peculiar shape and formation (chromophile cortex, chromophobe medulla) as certain invertebrate Golgi bodies (insects especially).

Assuming that plant cells have a Golgi apparatus, these platelets are the only things which could be a Golgi apparatus, because neither the

As the discovery of elements which we believe will soon be universally accepted as the Golgi bodies of plants, Bowen's work is interesting enough, but it is the special bearing which this discovery has on the 'vacuome' hypothesis of Guilliermond and Parat which is of value from the broader aspect of the mechanism of the living cell.

It is not the purpose of this paper to give a long discussion on the previous work of Guilliermond, Mangenot, and other plant cytologists.

We do not propose to enter into the question of the origin of the plasts, or the formation of various substances, such as starch, from plasts. In order to avoid such debatable points we have confined ourselves for the present as much as possible to the description of the various categories of cytoplasmic inclusions which can be found in plant cells treated by some of the techniques at present widely used by animal cytologists.

We have studied various plant tissues by methods which are known to reveal, in prepared sections, cytoplasmic inclusions which are visible intra vitam in animal cells. We have worked chiefly with the Champy OsO_4 method, usually known as the Kolatchev technique, which is par excellence the most reliable of all the chrome-osmium techniques. We have used the Mann-Kopsch (Weigl) method, but the figures and descriptions in this paper refer to the Kolatchev method. This technique is considered by the senior author as the most reliable osmium technique, for the following reason. The material is fixed in Champy's fluid, which is really only a Flemming-without-acetic to which bichromate of potassium has been added. This fluid has been used successfully for many years by one of us, and never causes precipitates if the washing out is

chondriome nor vacuoles of plant cells satisfy the conditions. The allegation of Guilliermond that the sinus-like vacuolar system is a Golgi apparatus is unacceptable, and has gained no ground.

Bowen has shown that in moss androgonia the platelets behave like the dictyosomes of an animal spermateleosis (see p. 391, Text-figs. 6 to 9 of this paper, and compare with any invertebrate spermatid).

carried out properly. The plant tissues are, after fixation, washed overnight under a running tap, and all of the free chrome salts are easily removed. The material is then transferred to 2 per cent. OsO_4, and incubated at 32° C. for four days. Afterwards the tissues are washed overnight under the tap, to remove any crystals of OsO_4. The advantage of the previous impregnation of the tissues in chromium trioxide and bichromate of potash, is that the reduction of the OsO_4 is slow and easily controlled. One hardly ever gets a bad precipitation of osmic crystals, as may occur with the corrosive-osmic method of Weigl.

The Mann-Kopsch or Weigl method we consider suffers from the fact that it is not possible to be certain that the corrosive sublimate is thoroughly washed out before the post-osmication. In fact we are inclined to consider that in plant tissues the inability of the corrosive sublimate to pass out through the thick cellulose walls will always be a fruitful source of artefact production. We have found ' vacuomes ' such as drawn by Bowen, but since we believe that many of these blackened areas are corrosive osmic artefacts, we have refrained from drawing any such cells in Pl. 25. Those cells we have drawn are regarded by the senior author as free from all suspicion of containing artefacts produced by the interaction of free corrosive and osmic crystals.

It will be found by the reader that we agree with most of Bowen's interpretations, though in a few unimportant points we find his statements obscure.

It should be mentioned that there is much in the previous work of N. H. Cowdry (**5**) and Mottier (**10**) with which we agree; such as for instance the morphology of the plastid and mitochondrial systems. The 'canalicular system' of Bensley (**3**) we have also found, but we do not wish to examine this question very deeply in the present paper. There appears to be much in the work of plant cytologists precedent to Bowen which will stand the test of more recent examination, the chief points of Bowen's work and this present study being the identification of a new system which the older workers have overlooked or failed to demonstrate.

PREVIOUS WORK.

Guilliermond (7, 7a, 8) and the French school of workers, who have been studying plant cytology for a considerable time, consider 'les deux seules entités morphologiques de toute cellule végétale (Guilliermond) et animale sont le vacuome (Dangeard) et le chondriome' (Parat and Painlevé, **11**).

From part of the chondriome (mitochondrial constituents) in plant cells, they find the plastidome (plastid system) to be derived, while the vacuome, in animal cells, forms the Golgi apparatus. These observers did most of their work with intra-vitam stains, neutral red and Janus green, but even in osmic acid preparations they found only the two above-mentioned classes.

Bowen has recently examined plant tissues with a view to clarifying our knowledge of the nature of the cell inclusions, and to discovering the homologues of animal cellular structures in the plant world.

He found four classes of structure in the plant cell (not including the nucleus and nuclear bodies).

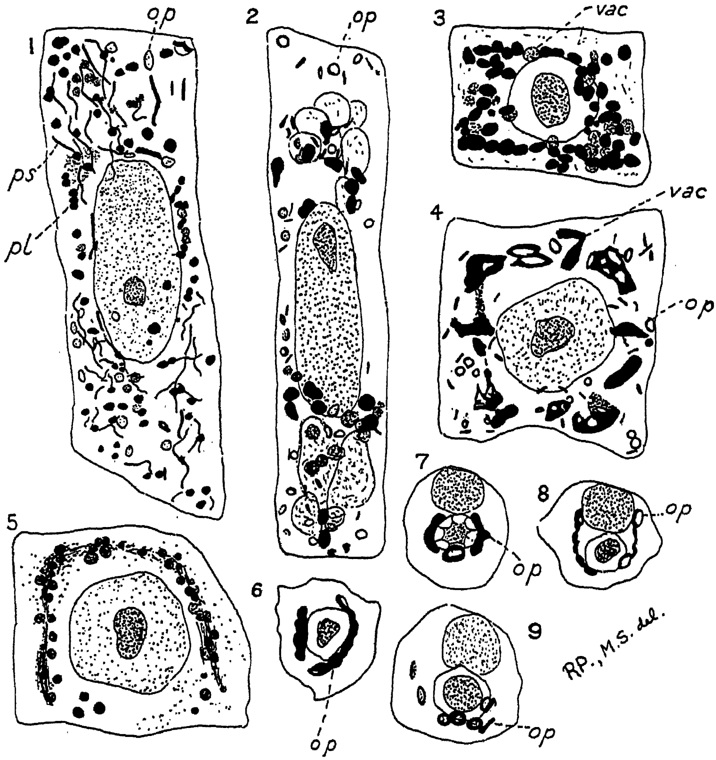
1. Pseudochondriome (possibly mitochondrial).
2. Plastidome (plastid system).
3. Vacuome (vacuoles).
4. Osmiophilic Platelets (possibly Golgi apparatus system).

He has observed these structures in members of the Spermatophyta, Pteridophyta, and Bryophyta. The chief methods which he has used are those of Kolatchev, Weigl, Hirschler, Champy-Kull, and Benda.

1. Pseudochondriome. Bowen found that for these structures the Champy-Kull method acted as practically a specific stain, but they were also shown up in root-tips of Vicia faba with the Kolatchev method. Text-fig. 1 shows the pseudochondriome as densely blackened spherical granules scattered irregularly through the cytoplasm; in older cells they sometimes showed signs of elongation. He found no evidence for Guilliermond's theory that the plastidome is derived from

the chondriome. In young androgonia of Polytrichum, the pseudochondriosomes are seen as in Text-fig. 5, in later stages of the androcytes they appear as threads encircling the nucleus ;

TEXT-FIGS. 1-9,



after Bowen. For description, see pp. 390–393.

this mass of threads then spreads out over the nucleus along the length of the sperm.

2. Plastidome. Bowen considers that this is a distinct element of the cell ; it is best demonstrated in Benda preparations. The proplastids as seen in Text-fig. 1 are thread-like. In later stages they become oval or rounded. Bowen has traced all

the steps in the deposition of starch in the plastid primordia. He found that they were orientated in a definite manner in cell division.

3. Vacuome.—Bowen found that this was best demonstrated in the root-tip by means of the Weigl method (corrosive osmic or Mann-Kopsch); he has not, as yet, found any trace of a vacuome in moss androcytes. The manner of impregnation appears to be very variable. This vacuome is shown in Text-figs. 2, 3, and 4. The vacuome is composed of small spherical vacuoles as in Text-fig. 3; these 'gradually merge together until the large vacuole characteristic of plant cells emerges'. Sometimes 'only more or less well marked traceries of black or grey (Text-fig. 2) mark the vacuoles'. Text-fig. 4 shows, in Vicia, a network developed by fusion of the primary vacuoles.

4. Osmiophilic Platelets.—Bowen has recently published a separate paper on his work on these structures which seem to have been completely overlooked by all previous workers. They show the appearance of ring-shaped structures as in Text-figs. 1, 2, and 4. They have been found present, and of practically similar appearance, in all material so far investigated. They are best demonstrated by the Kolatchev method. In cell divisions the platelets are fairly evenly distributed to the two daughter cells. Bowen says, 'the mode of multiplication of the platelets remains unsolved', but it appears probable that it is by fragmentation; not infrequently one finds groups of several small platelets which suggest an origin from a single original large one.

In the moss androcyte the osmiophilic platelets are found to be very large. They gather together along one side of the nucleus and the limosphere[1] appears in the midst of them; as the latter grows the osmiophilic platelets become scattered (Text-figs. 6–9). Bowen says, 'the relation of these platelets to the developing limosphere suggests in every way the formation of the acrosomal material in those animals in which the Golgi bodies do not fuse to form a single permanent mass—the acroblast'.

---

[1] See Wilson, 'Annal. Bot.', vol. 25, 1911.

Owing to the behaviour of osmiophilic platelets in spermatogenesis and to their remarkable similarity to the Golgi bodies of insects and many invertebrates, and for other reasons which he quotes, Bowen is convinced that these platelets are the homologues of the Golgi apparatus of animals.

MATERIAL AND METHODS.[1]

Material.	(*a*) Vicia	root-tips.
	(*b*) Pisum . . .	shoots.
	(*c*) Hyacinthus . .	root-tips.

No material was used which had been growing for more than four weeks, and only young growing root-tips and shoots were employed. The hyacinth bulbs were grown in tap-water in vases, a little charcoal being placed in the water to keep it pure.

In most cases the beans and peas were grown in moist sphagnum moss, but some of the former were grown in moss-fibre in a hot-bed (temperature 65° F. approx.).

Methods.—The ordinary procedure for Bouin's method and Da Fano's silver nitrate method was followed, but without success.

The osmic acid methods of Champy-Kull, Benda, Kolatchev, and Weigl were used. The Kolatchev method was found to be far the most effective, showing the platelets very distinctly. Details of the process are as follows :

(i) Fixed in Champy's fluid for 24 hours. In some cases 2 or 3 drops of pyrogallic acid were added to the Champy's fluid. The mixture was always fairly fresh.

(ii) Washed under the tap overnight.

(iii) Placed in 2 per cent. osmic acid at 32° C. 3–4 days. The capsule containing the material was placed in a large dish in which was a little water to prevent over-evaporation of the osmic acid.

(iv) Washed in running-water 24 hours.

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[1] The preparations used in this work were made by R. Patten and M. Scott.

(v) Upgraded through the alcohols.

(vi) Embedded in wax.

(vii) Sectioned thickness 2·5 μ.

The Weigl Method was also used, the details being followed as given by Bowen.

(i) Fixed in corrosive-osmic:

1 per cent. osmic acid	1 part
Saturated solution of mercuric chloride in 75 per cent. sodium chloride solution	1 part

The periods of fixation were ½, 1, 3¾ hours, but difference of time seemed to have no effect on the results.

(ii) Washed in running-water 30 minutes.

(iii) Placed in 2 per cent. osmic acid at 32° C. and treated as for Kolatchev.

In both these methods the only way to know whether the osmic acid has impregnated sufficiently is to tease out portions under the microscope from time to time.

Bleaching.—In some cases after the wax had been removed from the sections the slides were placed in turpentine for 1–12 hours. The action was checked from time to time under the microscope. The turpentine was then washed off in xylol and the slides mounted in balsam.

Occasionally the hydrogen peroxide method was used. The slides were brought down to water and then placed in H_2O_2 for about 1 minute. The action being watched under the microscope since bleaching occurs very rapidly.

In some cases bean root-tips were centrifuged previous to fixation. The centrifuge revolving for 1 hour at the rate of 3,000–4,000 revolutions per minute. The radius of revolution was 5½ in., thus producing an acceleration of 2,000 times gravity.

At first we cut the root-tips into very small pieces thinking this was necessary to allow thorough impregnation by the osmic acid; but when this was done it was found very difficult to embed them satisfactorily for cutting, so on all later occasions root-tips of not more than 1 cm. in length were placed whole in the fixative, and it was found that the osmic acid penetrated the cell walls quite easily. Sections were usually cut longitudinally.

Observations on Living Cells.—We have observed intra vitam the very granular protoplasm of the root-tip cells without being able to differentiate the different systems herein described. Our attempts to stain a 'vacuome' by means of neutral red were also uniformly unsuccessful, both in teased preparations and in plants grown in neutral-red fluid.

We are certain that, unless a vital and specific stain for Golgi bodies is discovered, it will not be possible to observe these plant Golgi bodies in living cells, because of the extremely numerous granules of the other systems. A specific intra vitam stain for Golgi bodies has not yet been forthcoming.

ORIGINAL OBSERVATIONS.

It has been mentioned that the present descriptions are of Kolatchev material. All osmic Golgi methods, when applied to animal tissues, tend to impregnate both Golgi elements and mitochondria. It is our experience that this applies more to the chrome-osmic methods (Kolatchev, Nassonow) than to the corrosive-osmic methods (Mann-Kopsch or Weigl). Thus all the cells drawn in Pl. 25 are from Kolatchev slides.

In our experience it is rare to get a Kolatchev preparation of mollusc gonad in which the mitochondria, as well as the Golgi bodies, are not consistently darkened. On the other hand the Mann-Kopsch method is usually much more specific. Thus our experience with plant tissues treated with chrome-osmic methods resembles that which we have had with animal material.

We find cells such as that of the bean root-tip, in which the mitochondria alone are shown (fig. 1, Pl. 25), or again more usually cells in which the osmiophilic platelets (figs. 2, 5, and 6, Pl. 25) alone are demonstrated. We may now describe the various types of inclusions which we have found.

1. In fig. 1, M, Pl. 25, are what the animal cytologist would unhesitatingly identify as mitochondria. Such granules have previously been drawn by N. H. Cowdry, E. V. Cowdry,

Mottier, Guilliermond, and many other cytologists. We do not think that there will be any likelihood of future workers quarrelling with this interpretation. This system is known to most continental observers as the chondriome. We prefer to call them mitochondria. Now in Kolatchev preparations cells which show the mitochondria best are found towards the outside of the root-tip. This is usually the case also in Kolatchev preparations of animal gonads. In fig. 7, Pl. 25, is another cell (root-cap) showing elongated elements which we believe are chondriomites. They are less common than the chondriosomes.

2. We find cells, such as that drawn in fig. 4, Pl. 25, in which much larger irregular elements are seen, marked P. These have previously been identified as plasts, and have been correctly figured by Mottier. In such cells one often seens mitochondria (M) as well, and we remember that the plasts have been described by various authors as being derived from the mitochondria. In other cells (fig. 3, Pl. 25) we can see the same type of plast, together with osmiophilic platelets (OP). These plasts form the plastidome system of continental workers. As has been mentioned above, Bowen does not agree with Guilliermond that the plastidome is derived from the chondriome system.

3. The osmiophilic platelets are shown alone impregnated in figs. 2, 5, and 6, Pl. 25, and they form by far the commonest cytoplasmic inclusion revealed by the Golgi osmic methods. At a certain level in the plant tissue, just as at a certain level in some animal tissue treated by Kolatchev, only the Golgi elements are impregnated. These osmiophilic platelets are very small, but in good preparations impregnate so clearly on a yellowish protoplasmic background that their structure can be made out very easily. They are certainly disks or platelets, because when seen edgeways they appear as a black line. Seen flatwise they are found to be formed of an osmiophile cortex and a central chromophobe medulla. They rarely form chains, and do not unite when lying close together. Where an individual platelet lies in a protoplasmic bridge of a smaller bore than the width of the platelet, the latter keeps its shape.

The platelet is therefore of a greater viscosity or solidity than protoplasm.

No evidence of the platelets swelling up in any way has been found. The platelets are pretty uniform in size in the same cell. In the various plants we have studied they do not vary in size very much.

We were unable to shift them with a powerful centrifuge. We have not succeeded in demonstrating them with F.W.A., Champy haematoxylin, or the silver-nitrate Golgi methods, though since formalin gives such a poor fixation of plant cells, this is not to be expected. We have not tried the effect of modifying the Da Fano fixative.

4. Vacuolar System.—As far back as 1910 Bensley described a vacuolar or canalicular system in root-tip cells. Systems of spaces or vacuoles, occasionally containing an osmiophile or argentophile substance, have since been described by Guilliermond and his pupils.

Bowen in his 1927 paper describes a ' vacuome ' in plants, and has suggested that the vacuoles have lipoid primordia, at least ' in the sense that the vacuolar walls were lipoidal in nature ' (Bowen, **1**, p. 187).

Now we have found that the Mann-Kopsch (corrosive-osmic) method reveals a system of blackened spaces in the cells of the bean root-tip, but since we have been unable to satisfy ourselves that these spaces correspond with the pictures given by Flemming-acetic, Bouin, and Kolatchev methods, we do not believe that they are completely above suspicion. This is a part of Bowen's work which is not so satisfactory as his descriptions of other inclusions, and Bowen himself leaves the matter in an unsettled state.

Possibly there is a slight tendency for Bowen (and we have ourselves experienced this) to be influenced by the view that the ordinary intercellular spaces or vacuoles are necessarily a ' system ' homologous with the vacuolar system associated with the Golgi elements of animal cells.

One of us (J. B. G.) is of the opinion that no ' system ' has been demonstrated in plant cells. By 'system' we mean self-perpetuating primordia and specifically staining, which can be found in the same state in all cells of the same kind and stage of development.

In figs. 1, 2, 4, 5, and 6, Pl. 25, are cells containing intracellular spaces or vacuoles. There is no evidence whatever that these spaces are homologous with the 'vacuome' of animal cells, or with the nerve-cell Golgi apparatus, as suggested by Guilliermond and Parat.

We are aware that bacteria exist in the roots of leguminous plants, and are familiar with the paper of E. V. Cowdry (**4**) on the bacteria and mitochondria of the root-nodules of such plants. We do not believe that any of the elements we have described resemble the bacteria found in the older roots described by Cowdry.

We have also taken the precaution of examining the shoots of the pea, and have found cytoplasmic inclusions similar to those depicted in figs. 1–7, Pl. 25.

THE SUPPOSED PLANT-CELL GOLGI APPARATUS OF A. H. DREW.

In the 'Journal of the Royal Microscopical Society' of 1920, Dr. Audrey Drew has given a description of certain bodies in plants which he identified at that time as Golgi elements. Reference to Drew's paper and figures shows that his Golgi bodies are the same structures as are called plastids by Mottier, Guilliermond, Bowen, and ourselves, and which are figured in the present paper in fig. 4, Pl. 25, especially; this figure closely resembles his fig. 3 and Mottier's figures.

We consider that the structures identified as Golgi bodies by Dr. Drew are the plastidome system.

DISCUSSION.

Guilliermond, Parat, and Painlevé consider that two systems exist in plant cells—chondriome and vacuome. It will be seen that Bowen has demonstrated clearly that a third system—the osmophilic platelets—exists.

We consider that three systems have been shown to exist in plant cells, chondriome (mitochondrial contents) ; plastidome (plastid system), and Golgi elements (osmophilic platelets); not found by Guilliermond. The ' vacuome ' or vacuolar system, the homologue of the ' vacuome ' of animal cells as claimed by Guilliermond and Parat, appears to be only the usual intercellular vacuoles, which are common in plant cells. There is no evidence that the plant vacuoles are ever associated with Golgi elements as in animals.

Difference between Animal and Plant cells.—The cells of animals and the higher plants agree in the presence of mitochondria and Golgi elements. The plant Golgi elements resemble those found especially in the Hemiptera among animals.

Discoidal mitochondria have, however, been described by Gatenby and Bhattacharya in scorpions (**6**).

In plants only one additional cell element is at present known to exist—and this the plast. Bowen considers that the plasts are not derived from the chondriosomes as has been widely believed by continental workers. We are not so sure that Bowen is right, and we are inclined to believe that the plastidome is merely formed by enlarged mitochondria as Guilliermond, Mottier, and others have suggested.

If such is the case there is no basic difference between the cytoplasmic equipment of plant and animal cells. It is true that the Golgi elements of plant cells are more numerous than is usual in animal cells, and that they have not been described in the typical juxtanuclear position. As, however, nearly all published work in the plant Golgi elements refers to vegetative cells, e.g. root and shoot cells, we must await further studies on the reproductive and embryonic cells.

It has been established that the plant cell, as well as the animal cell, contains a Golgi system. It is too soon to speculate on the general morphology of the Golgi apparatus of plants, but we are now certain that the animals and plants are derived from an ancestor which already had differentiated a cytoplasmic system with both mitochondria and Golgi bodies.

From the labours of Bowen it appears that in the androgonia of mosses at least the Golgi bodies serve the same function in plants as in animals. In many tissue cells the Golgi elements are scattered as in the vegetative cells of plants, but in no vegetative cells known yet is the peculiar juxtanuclear excentric position of the animal Golgi apparatus found.

June 25, 1928.

SUMMARY.

1. The plant cell has the following cytoplasmic inclusions :

(*a*) Mitochondria (chondriome).

(*b*) Plastids (plastidome), probably derived from mitochondria.

(*c*) Golgi elements (osmiophilic platelets of Bowen).

2. Plant cells often contain vacuolar spaces filled with a watery fluid. These spaces are sometimes canalicular in arrangement, as shown by Bensley eighteen years ago.

3. There is no evidence that these vacuoles are :

(*a*) Formed from self-perpetuating primordia.

(*b*) Possessed of a lipoid membrane.

(*c*) Associated with the Golgi elements (platelets).

4. There is no reliable evidence that the plant-cell cytoplasm contains any type of protoplasmic inclusion not also found in animal cells, for the plasts are probably enlarged mitochondria, as has been suggested by Mottier, Guilliermond, and others.

5. The so-called ‘ vacuome ’ drawn by Bowen (this paper, p. 391) is found almost always in Weigl (Mann-Kopsch) preparations. Such ‘ vacuoles ’ and primordia are possibly corrosive-osmic artefacts caused by the non-ability of water to wash out the corrosive sublimate of the Mann's fluid, previous to osmication.

6. The osmiophilic platelets are demonstrable by the Kolatchev and Mann-Kopsch methods, but we have not so far succeeded in showing them with Benda, Flemming-without-acetic, haematoxylin, Champy-haematoxylin, or the silver-nitrate Golgi methods. They resemble closely the dictyosomes of Hemipterous germ cells.

LITERATURE.

1. Bowen, R. H.—" Preliminary Report on the Structural Elements of the Cytoplasm in Plant Cells ", ' Biol. Bull. of Marine Biol. Laborat.', vol. liii, no. 3.
2. —— " Studies on the Structure of Plant Protoplasm ", ' Zeit. f. Zellf. u. mikr. Anat.', Bd. 6, Heft 5, 1928.
3. Bensley, R. R.—See ' General Cytology ' (E. V. Cowdry), p. 343.
4. Cowdry, E. V.—" Independence of Mitochondria and the Bacillus Radicicola in Root Nodules ", ' Amer. Journ. Anat.', vol. 31.
5. Cowdry, N. H.—" A Comparison of Mitochondria in Plant and Animal Cells ", ' Biol. Bull.', vol. 33, pp. 196–228.
6. Gatenby, J. Brontë, and Bhattacharya, D. R.—" Notes on the Cytoplasmic Inclusions in the Spermatogenesis of the Indian Scorpion ", ' La Cellule ", vol. xxxv, 1924.
7. Guilliermond, A.—" Nouvelles recherches sur les constituants morphologiques du cytoplasme de la cellule végétale ", ' Arch. d'Anat. Microscop.', vol. 20 (1926).

7 *a*. —— "Sur l'action des méthodes à imprégnation osmique sur les cellules végétales ", ' C. R. des Séances de la Soc. de Biol.', vol. 95.

8. —— " Sur l'origine mitochondriale des plastides ", 'C. R. Acad. Sci. Paris ', 167 : 430–3 (1918).
9. —— " Nouvelles recherches sur l'appareil vacuolaire dans les végétaux ", ibid., 171 : 1071–4 (1920).
10. Mottier, D. M.—" Chondriosomes and the Primordia of Chloroplasts and Leucoplasts ", ' Ann. of Bot.', vol. 32.
11. Parat, M., and Painlevé, J.—" Appareil réticulaire interne de Golgi, trophosponge de Holmgren et vacuome ", ' C. R. Acad. Sci.', October 1924.

DESCRIPTION OF PLATE 25.

All Kolatchev technique.

LETTERING.

M, mitochondria ; P, plastid ; OP, Golgi element (osmiophilic platelet).

Figs. 1, 2, 3, 4, and 7, the bean (Vicia faba) ; figs. 5 and 6, the hyacinth (Hyacinthus).

Fig. 1.—Root-tip cell, showing mitochondria.

Fig. 2.—Ditto, showing osmophilic platelets.

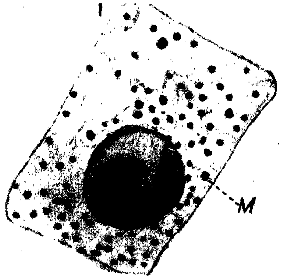
Fig. 3.—Ditto, showing plastids and platelets.

Fig. 4.—Ditto, showing plastids and mitochondria.

Figs. 5 and 6.—Ditto, showing platelets.

Fig. 7.—Root-cap cell, showing filaments supposed to be chondriomites (elongated or fused mitochondria).

1



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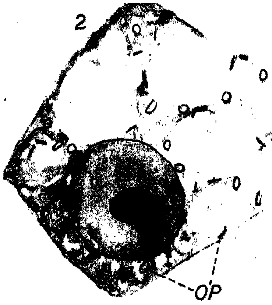

Figs. 1, 2, 3, 4, & 7 10μ

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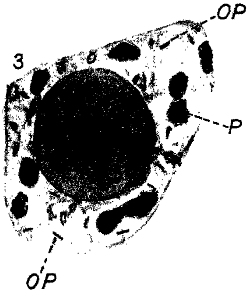

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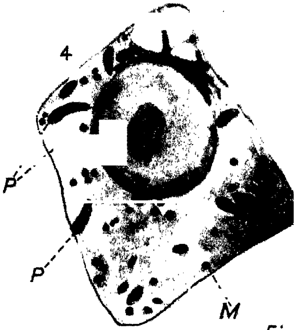

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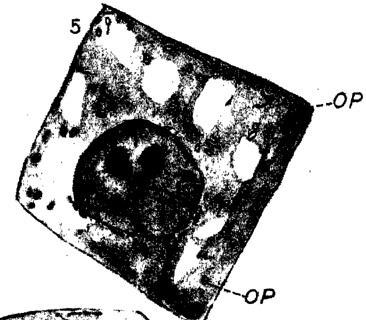
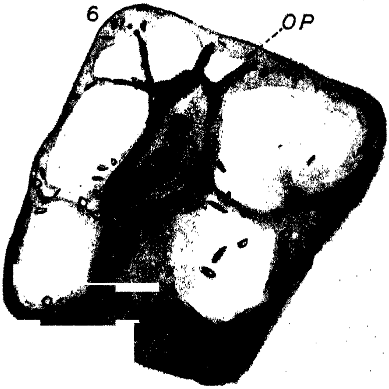


Figs. 5 & 6 10μ

6



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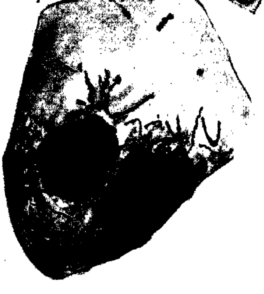

Patten, Scott, and Gatenby, del.

Studies on the Origin of Yolk.
II. Oogenesis of the Scolopendra, Otostigmus Feae (Pocock).

By

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and

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With 11 Text-figures.

IN a previous paper (1924) on the egg of the centipede, Lithobius forficatus, one of us (V. N.) described two kinds of yolk, albuminous and fatty. The albuminous yolk is preceded by nucleolar extrusions of a remarkable type, and its origin seems to be associated with them, although no evidence could be adduced that they are directly transformed into the yolk. It was further shown that the juxta-nuclear Golgi apparatus fragments into small granules and small crescent-shaped Golgi elements. The former grow in size and give rise to the fatty yolk. Miss S. D. King (1924) confirms the above account of the association of the albuminous yolk with the nucleolar extrusions, but in her opinion this type of yolk arises directly from these extrusions. With regard to the Golgi apparatus she admits that it fragments into small granules. Furthermore she describes fatty yolk :—'the origin of this fatty yolk is doubtful, but it may possibly be connected with the Golgi apparatus, although no evidence in support of this theory has been discovered'. In our opinion Miss King is doubtful of the origin of fatty yolk from the Golgi apparatus, because 'both Mann-Kopsch and Da Fano material was studied, but the latter gave such favourable results that it was used almost exclusively' (spaced words ours). Now it is well known that the Da Fano method fails to show fat in the finished slides, while the Golgi apparatus is preserved. On the other hand the Mann-Kopsch method preserves both Golgi apparatus and fat, and thus enables one to study the transformation of the former into the latter. Furthermore, Flemming-without-acetic, which Miss King has used, shows fat, but not the Golgi apparatus, at least in the egg of Lithobius. In a later paper (1926), however, Miss King has shown that ' the formation of fatty yolk from the Golgi elements in Oniscus is strictly comparable to the process described by Nath in Lithobius. . . .'

In view of the above conflicting evidence it was thought desirable to work out the origin of yolk in the eggs of the centipede Scolopendra. Our observations, both on fixed and fresh material, unmistakably show the origin of the fatty yolk from the Golgi elements and furnish a strong confirmation of the same process in Lithobius. The nucleolar extrusions of Scolopendra are few and they disappear at a particular stage; and between their disappearance and the origin of albuminous yolk there is a long pause which eliminates all possibility of the direct origin of this type of yolk from these extrusions.

The routine laboratory technique employed is mentioned in the text and in the explanatory notes on the figures. One of us (V. N.) has also studied fresh material with the help of neutral red and janus B. All diagrams have been drawn by Vishwa Nath.

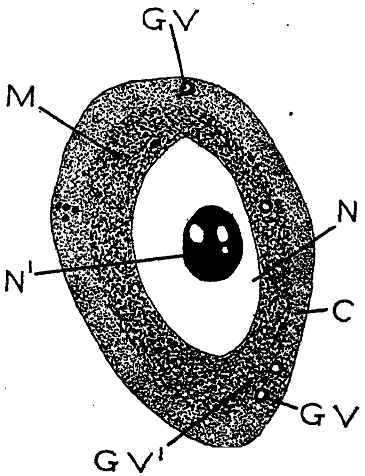
It is a very great pleasure to us to thank Colonel S. R. Christophers, F.R.S., Director of the Central Research Institute, Kasauli, who gave us all facilities for this work in his institute in the summer of 1926. Our thanks are also due to the authorities of the Indian Museum for identifying the Scolopendra and for supplying us with the necessary literature.

OBSERVATIONS.

Staining with vital dyes gave good results. The ovary is kept in neutral red or janus green B for about fifteen minutes. It is then mounted on a slide in a drop of the stain, covered with a cover-slip, and studied under the high power of the microscope. Text-fig. 9 represents a fairly young oocyte. The whole oocyte is full of very clear colourless vacuoles of similar size distributed in a uniform manner throughout the cytoplasm; these are the Golgi vacuoles. In the spaces between the vacuoles lie the granular mitochondria also distributed uniformly. Both the cytoplasm and the mitochondria are stained red or green according to the dye used. The mitochondria, however, stain deeper than the cytoplasm and appear more distinctly with janus green B than with neutral red. With the growth of the oocyte both the Golgi vacuoles and the mitochondria increase in number (Text-fig. 10). The mitochondria remain granular, but some of the Golgi vacuoles increase in size and give rise to the fatty yolk. Both the Golgi vacuoles and the fatty yolk-vacuoles appear as colourless vacuoles, but their contents are chemically different, as will soon appear. Excellent results are obtained when a most highly developed egg is stained with neutral red. Such an egg can be studied only under the lower power as the big size of the egg does not allow the use of the cover-slip. In Text-fig. 11 a portion of such an egg stained with neutral red is represented. It is studied under the low power and is magnified only forty times. Nevertheless appearances are brilliant. At *A.Y.* is the albuminous yolk which appears as colourless, solid, and homogeneous spheres. At *F.Y.* are the fatty yolk-vacuoles which appear brilliantly red as the stain has penetrated inside them. The smaller Golgi vacuoles, however, remain colourless. The granular mitochondria cannot be seen with this magnification. If now the egg is broken with a needle the solid albuminous yolk-spheres are seen scattered on the slide, but the vacuoles, both Golgi and fatty yolk, being delicate are ruptured and do not appear. In some cases, however, a small portion of the cytoplasm of the ruptured egg remains intact and the vacuoles can be seen, as if held in position by the solid albuminous yolk-spheres. On account of the paucity of material oocytes younger than that represented in Text-fig. 9 could not be studied with the vital dyes.

Text-fig. 1 represents an unstained Mann-Kopsch preparation of the youngest oocyte that we have been able to obtain. The mitochondria appear granular and brownish and form a complete ring round the nucleus. At *G.V.* are the Golgi vacuoles, each with a sharp black chromophilic rim and a central chromophobic substance. The contents of each vacuole are watery. At *G.V.′* the Golgi vacuoles appear solid on account of the precipitation of the metallic osmium inside their interior. With the growth of the oocyte the mitochondrial ring spreads out at its

TEXT-FIG. 1.



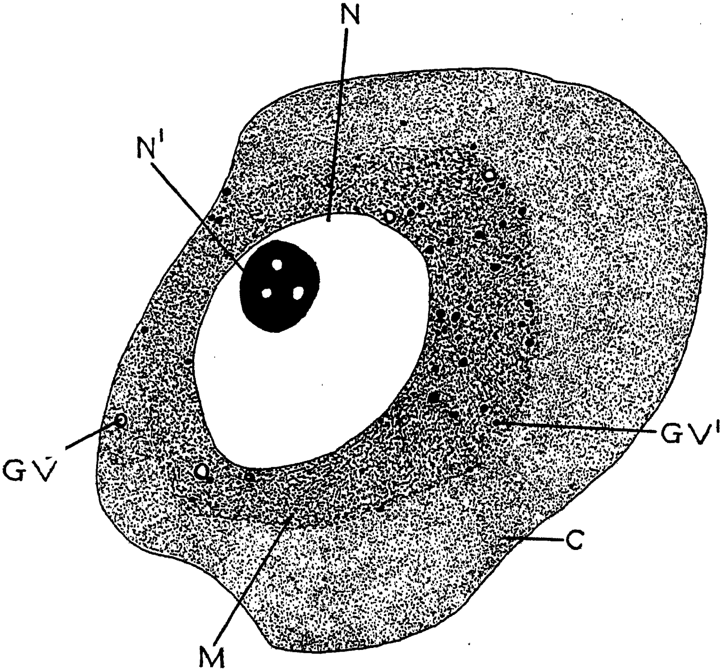
Youngest oocyte showing Golgi vacuoles and mitochondrial ring. Mann-Kopsch unstained. ×2,100.

EXPLANATION OF LETTERING OF TEXT-FIGURES.

AY, albuminous yolk; *C*, cytoplasm; *FY*, fatty yolk; *GV*, Golgi vacuole; *GV′*, Golgi vacuole looking solid on account of excessive impregnation; *GV″*, Golgi 'crescent' with 'idiosome'; *GV‴*, Golgi 'crescent' without 'idiosome'; *M*, mitochondria; *N*, nucleus; *N′*, nucleolus; *N″*, secondary nucleoli; *NE*, nucleolar extrusions.

periphery and the Golgi vacuoles increase in number, as shown in Text-fig. 2 which represents a Mann-Kopsch preparation stained with acid fuchsin. In Text-fig. 3, which is a Mann-Kopsch unstained preparation, the mitochondrial ring has

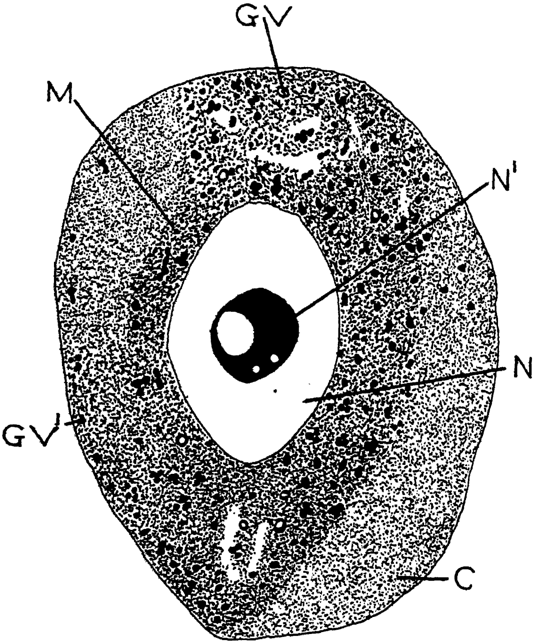
TEXT-FIG. 2.



Slightly older oocyte showing proliferation of the Golgi vacuoles and the spreading out of the mitochondrial ring. Mann-Kopsch stained. ×1,800.

spread out and is touching the egg-membrane at the two poles. The Golgi vacuoles have further increased in number and most of them look solid for the reason given above. With the further growth of the oocyte a change takes place in the contents of the Golgi vacuoles. Text-fig. 4 represents a Champy-Kull preparation stained with acid fuchsin. At *G.V.* are the Golgi vacuoles which are stained with acid fuchsin. Each vacuole shows a red rim and a clear central substance. The contents of these vacuoles are undoubtedly watery and non-fatty. At *F.Y.* are shown vacuoles whose contents have become fatty. In unstained Champy-Kull preparations these vacuoles appear solid and dull black on account of the coagulation and osmication of the fatty contents by osmic acid. But when the slide is heated with the acid fuchsin stain floating on it, these solid and dull black bodies appear as clear vacuoles on account of their decolorization by acid fuchsin aided probably by heat. How acid fuchsin which is dissolved in anilin-oil water decolorizes osmicated bodies we cannot explain. It is, however, certain that acid fuchsin tends to decolorize osmicated bodies in much the same way as turpentine and xylol. It will be noticed that in Text-fig. 4 the number

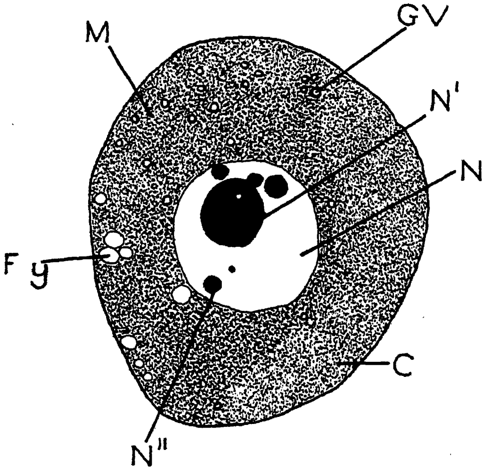
TEXT-FIG. 3.



Older oocyte showing the same phenomenon. Mann-Kopsch unstained. ×1,680.

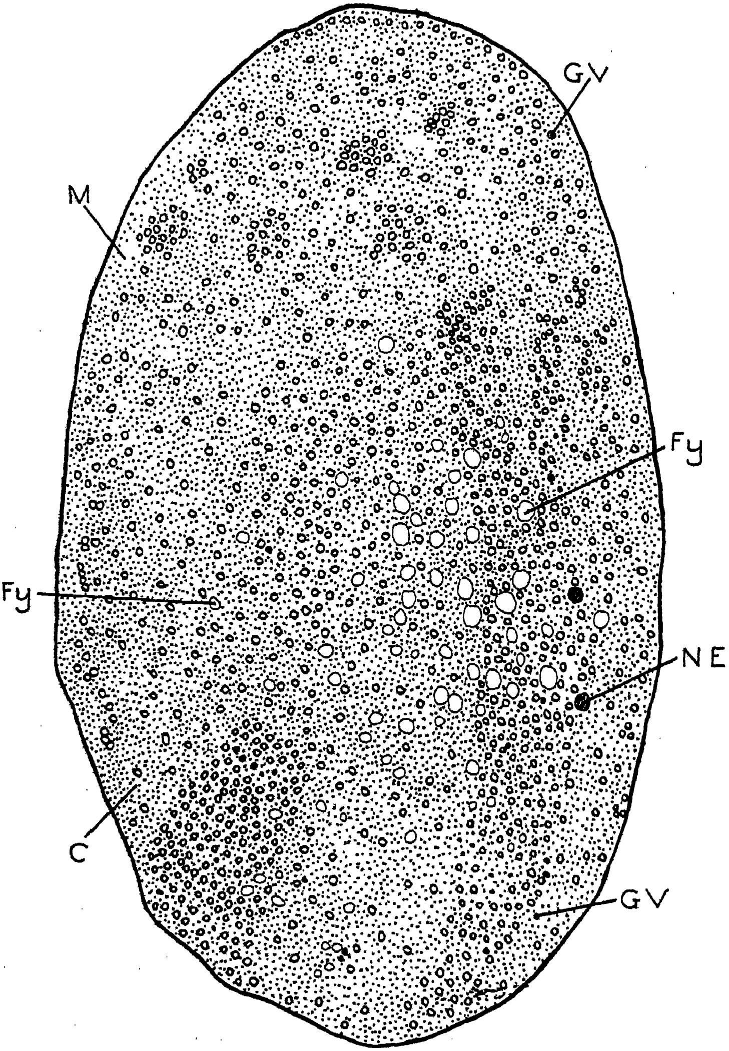
of Golgi vacuoles is much smaller than in Text-fig. 3, although the latter represents a younger oocyte. This, however, is due to the Champy-Kull method which is certainly inferior to the Mann-Kopsch method so far as the fixation of the Golgi vacuoles with watery contents is concerned. Text-fig. 5 represents an oocyte of a later stage fixed with Mann-Kopsch and stained with acid fuchsin. This is one of the best preparations in our possession as it comes very near to fresh eggs studied with vital dyes. The whole oocyte is full of Golgi vacuoles, each having a sharp black rim and a central clear substance. The uniform arrangement of these vacuoles that we find in fresh eggs (Text-fig. 10) is disturbed undoubtedly by the action of the fixative. At *F.Y.* are the fatty yolk-vacuoles which are bigger than the Golgi vacuoles. In unstained preparations they appear solid and dull black on account of the coagulation and osmication by osmic acid, but when the slide is stained with acid fuchsin they appear as colourless vacuoles without a sharp black chromophilic rim

TEXT-FIG. 4.



A young oocyte showing Golgi vacuoles, fatty yolk-vacuoles, mitochondria, and nucleolar budding. Champy-Kull stained with acid fuchsin. × 920.

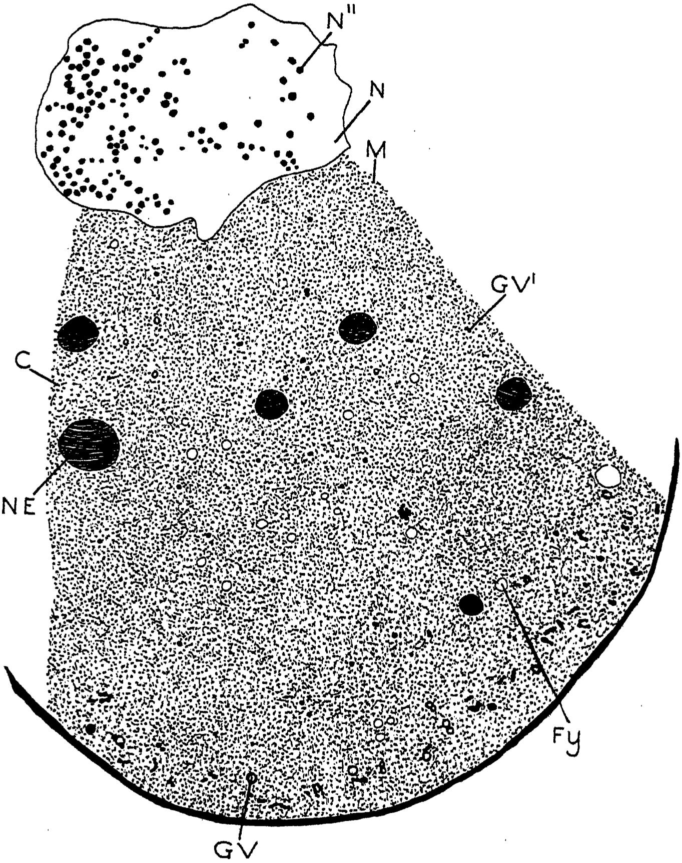
TEXT-FIG. 5.



An oocyte showing Golgi vacuoles, fatty yolk, mitochondria, and two nucleolar extrusions. Mann-Kopsch stained. × 300.

of the Golgi vacuole. The mitochondria remain granular and henceforth are uniformly distributed throughout the cytoplasm.

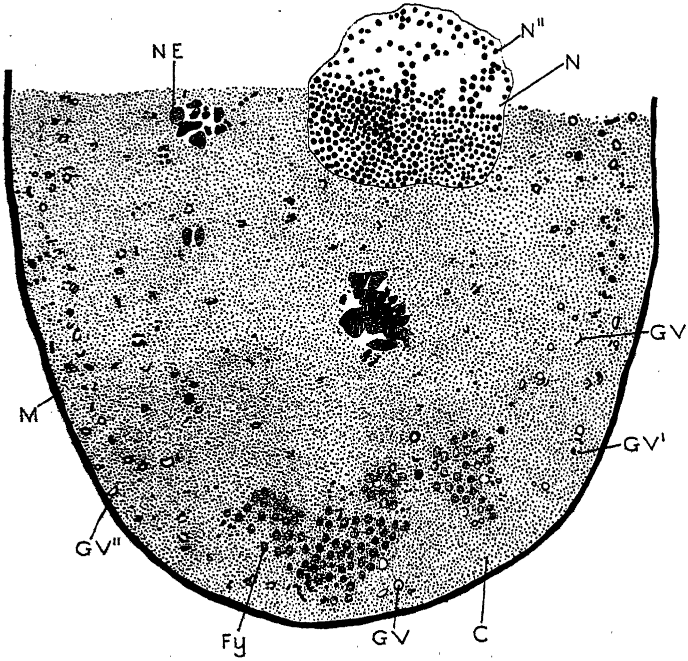
TEXT-FIG. 6.



Older oocyte showing the failure of OsO_4 to impregnate all the vacuoles. Mitochondria and nucleolar extrusions are also shown. Mann-Kopsch stained. ×550.

Text-fig. 6, which is a stained Mann-Kopsch preparation, represents an oocyte of a later stage than that represented in Text-fig. 5. This figure is drawn to show that osmic acid sometimes gives unsatisfactory results without any apparent reason. When compared with Text-fig. 5 it will be seen that many Golgi vacuoles have missed impregnation, as is shown by a smaller number of these elements in this diagram, although the fresh eggs of this stage are full of these vacuoles. In addition to this failure of OsO_4 to impregnate the Golgi vacuoles, the acid may cause certain artefacts. The artificial solid appearance of these vacuoles on account of excessive impregnation has already been mentioned. But osmic acid may incompletely impregnate a Golgi vacuole. In Text-fig. 7, which is a stained Mann-Kopsch preparation, certain crescent-shaped Golgi elements are shown.

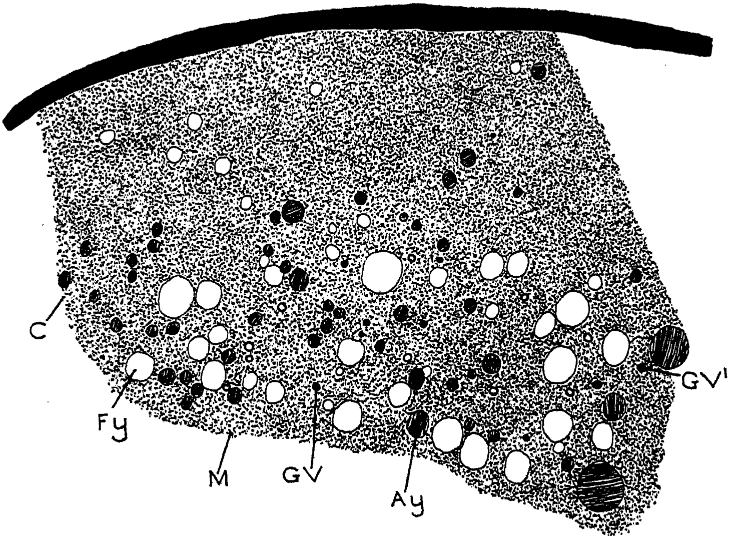
TEXT-FIG. 7.



Older oocyte showing the failure of OsO_4 to impregnate the Golgi vacuoles completely. Hence result 'crescents' with or without idiosome. Fatty yolk solid and dull black. Nucleolar extrusions degenerating. Mann-Kopsch stained. ×270.

Some of these crescents at *G.V.*″ have a clear substance attached to them which has been described as the idiosome or the archoplasm by various workers. Other crescents, however, may have no clear substance attached to them, e.g. at *G.V.*″ It is certain that these appearances are artefacts due to the osmication of a portion only of the ' wall ' of the vacuole. We do not see these crescents in Text-fig. 5 which we consider to be an ideal preparation, and what is more important they are entirely absent from fresh eggs. We are not prepared to make the general statement that crescent-shaped Golgi elements are never met with in any type of cell, especially when such competent workers as Bowen and Gatenby have consistently described them in the sperm-forming cells of insects, &c. We shall, however, at present content ourselves with saying that the solid granular type of Golgi element is an artefact wherever it has been described, due

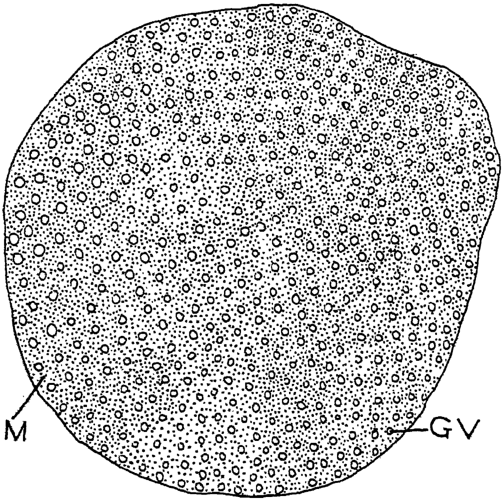
TEXT-FIG. 8.



Very old oocyte showing a few Golgi vacuoles and a number of fatty yolk-vacuoles. Albuminous yolk putting in its appearance. Mann-Kopsch stained. × 700.

to the excessive precipitation of metallic silver or osmium, and that crescents of the egg of Scolopendra at least are also artefacts due to incomplete impregnation. On the other hand, the crescents may represent only the optical sections of osmicated Golgi vacuoles. In the same figure at *F.Y.* are the fatty yolk-bodies which appear solid and dull black in spite of the fact that the slide has been stained with acid fuchsin. The inferiority of this preparation is made evident not only by the artefacts described above but also by the presence of fewer Golgi vacuoles and fatty yolk-vacuoles as compared with Text-fig. 5, although the latter represents a younger oocyte.

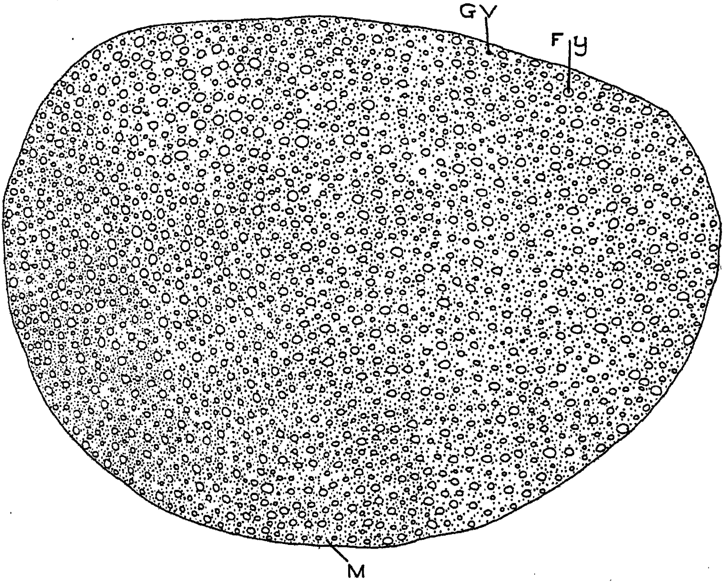
TEXT-FIG. 9.



A young oocyte stained with neutral red showing Golgi vacuoles of uniform size and granular mitochondria. Nucleus out of focus. ×210.

The origin of the albuminous yolk is very interesting. Long before the mitochondria and the Golgi elements get uniformly dispersed in the cytoplasm the nucleolus shows signs of budding (Text-fig. 4). The number of these nucleolar nucleoli quickly increases and some of them pass into the cytoplasm and grow in size (Text-fig. 6). Although there are numerous nucleoli, nucleolar extrusion are few. At first sight it appeared to us that these extrusions give rise to the albuminous yolk, since histochemically they are very much like the latter and also because they precede them. Study of later stages, however, soon showed that these extrusions disappear long before the albuminous yolk puts in its appearance. The process of their disappearance may thus be described. At first the extrusions swell up probably by absorbing some liquid from the cytoplasm, and then each one of them undergoes disintegration (Text-fig. 7, *N.E.*). The pieces that result from this disintegration are irregular in form and show obvious signs of degeneration. Gradually they disappear. The albuminous yolk arises de novo for the first time

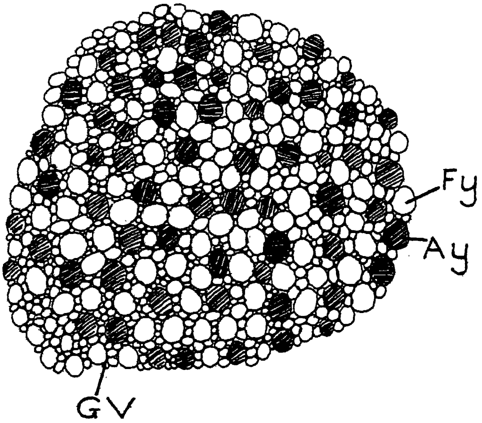
TEXT-FIG. 10.



An older oocyte stained with neutral red showing the growth of some of the Golgi vacuoles to give rise to the fatty yolk-vacuoles. Mitochondria granular. Nucleus out of focus. × 190.

when the egg has reached the dimensions represented by Text-fig. 8. The oocytes between the stages represented by Text-figs. 7 and 8 are devoid of any nucleolar extrusions, so that a direct origin of the albuminous yolks from the extrusions is out of the question. The possibility remains, however, that when the extrusions disappear their material might contribute towards the formation of albuminous yolk, but there is no way of ascertaining this. New albuminous yolk-spheres arise rapidly till we get the most highly developed ovarian egg (Text-fig. 11).

TEXT-FIG. 11.



A portion of the most highly developed egg stained with neutral red showing albuminous yolk, Golgi and fatty yolk-vacuoles. Mitochondria cannot be seen with this magnification. ×40.

DISCUSSION.

In support of our claim that the Golgi elements give rise to the fatty yolk we have shown that the latter puts in its appearance after the Golgi elements begin to increase in number and spread out in the cytoplasm, and that between the small Golgi vacuoles and the big fatty yolk-vacuoles there are vacuoles of intermediate dimensions. But above all we emphasize the fundamental morphological similarity between the Golgi elements and the fatty yolk, inasmuch as both are vacuoles.

This has been ascertained not only by good Mann-Kopsch preparations but also by the study of fresh eggs. It will thus appear that as in spiders (Nath, in press) and Lithobius (Nath, 1924) the Golgi vacuole, whose contents are watery and non-fatty, gives rise to the fatty yolk-vacuole by a process of growth and deposition in its interior of a colloid in the form of free fat not miscible with the general cytoplasm.

Although nucleolar extrusions clearly give rise to the albuminous yolk in various eggs, e.g. Saccocirrus (Gatenby, 1922), the Cockroach (Hogben, 1920), Lithobius (King, 1924, and Nath, 1924), and Luciola (Nath and Mehta, in press), &c., this is not the case in Scolopendra. The direct origin of this type of yolk from the nucleolar extrusions is impossible, as there is a long pause between the disappearance of the extrusions and the first appearance of the yolk; besides, nucleolar extrusions are very few.

SUMMARY.

1. The oogenesis of Scolopendra has been worked out with vital dyes and the routine laboratory technique.
2. The Golgi elements exist in the form of vacuoles with watery and non-fatty contents.
3. The solid Golgi elements are artefacts due to the excessive precipitation of metallic osmium inside the vacuoles.
4. The Golgi crescents are either artefacts due to incomplete blackening of a vacuole, or they are simply the optical sections of an osmicated vacuole.
5. The Golgi vacuoles give rise to fatty yolk-vacuoles by a process of growth and deposition inside them of free fat not miscible with the general cytoplasm.
6. The mitochondrial granules form a complete ring round the nucleus of the youngest oocyte. Later they are uniformly distributed throughout the cytoplasm.
7. There are a few nucleolar extrusions but they disappear long before the albuminous yolk puts in its appearance de novo in the cytoplasm.

BIBLIOGRAPHY.

1. Gatenby, J. Brontë (1922).—‘ Quart. Journ. Micr. Sci.’, vol. 66.
2. Hogben, L. (1920).—‘ Proc. Roy. Soc. London ’, Series B, vol. xci.
3. King, S. D. (1924).—‘ Scient. Proc. Roy. Dub. Soc.’, vol. 18.
4. —— (1926).—‘ Proc. Roy. Soc. London ’, Series B, vol. c.
5. Nath, V. (1924).—‘ Proc. Phil. Soc. Camb. Biol. Sciences ’, vol. i.

The Effect of a Temperature Gradient on the Early Development of the Chick.

By

Maria A. Tazelaar.

With Plate 26 and 4 Text-figures.

INTRODUCTION.

THE researches and theories of Child on the existence of 'Axial Gradients' have aroused considerable interest and speculation. Much of the evidence bearing on the problem has been obtained by means of the susceptibility of different parts of an organism to toxic solutions of varying strengths. Thus Bellamy (1919) demonstrated regions of high protoplasmic activity at the animal pole and dorsal lip of the blastopore in the frog's egg, which were repeated by Bellamy and Child (1924) after having been criticized by Cannon (1923). L. Hyman (1927) by subjecting chick embryos of varying ages to toxic solutions was able to demonstrate the presence of a simple antero-posterior death gradient in very early forms by means of 'differential disintegration'. The result in older embryos proved to be less simple, secondary gradients having been superimposed upon the primary antero-posterior gradient.

Geinitz (1925) observed that the axes of secondary embryos which were induced by 'organiser' implantations in Triton corresponded to that of their hosts. Ekman (1925), Stohr (1925), Copenhaver (1926), and Harrison (1918, 1921, 1925) have shown that the antero-posterior axis of the gills, the heart, and the limb-buds is the first to be determined.

If a developing organism could be subjected to a temperature gradient, which can be regarded as a graded stimulus, it might be possible to modify or even alter the original primary axial gradient. This method has been applied to frog's eggs by Huxley (1927) and continued by Shaw, Dean, and Tazelaar (1927).

Preliminary experiments using the same method have been made upon certain insect pupae, Calliphora vomitoria, and some Lepidoptera pupae. The effects observed upon the insects after emergence were remarkably small, especially as they had been treated for six hours per day for five days in succession. The changes in the segmentation of the frogs' eggs were striking in many cases, but later effects on the tadpoles proved slight.

Dareste (1877) applied unilateral heating in a way which resulted in a temperature gradient. He incubated eggs by placing them in contact with a tube through which warm water, apparently at the temperature of the hen's body, was flowing. By placing the egg in various positions relative to this tube, greater heat could be applied to the head, tail, right or left sides of the embryo. The whole apparatus was unenclosed, not contained in a regulated incubator as in our experiments, and there must have been considerable fluctuations in the temperature of the egg, with changes in the temperature of the room.

He often obtained asymmetrical blastoderms, with greater extent of blastoderm on the heated side. He also obtained several cases (on one occasion in every specimen of a treated batch of eggs) of heterotaxia (reversed flexure of the embryo) when the heat was applied to the left side. He therefore drew the conclusion that normally the right side of the embryo grew more than the left, and thus produced flexure. Dareste states that he obtained embryos with relative disproportion of parts owing to this treatment.

His views have been criticized by Warynski and Fol (1884). These authors applied strong radiant heat from a red-hot thermo-cautery needle held just above the surface, close to the left side, of embryos of 24–36 hours. The heat was well beyond the optimum, and caused marked inhibition of growth of the adjacent areas. However, they obtained several cases of heterotaxia when heat was applied in this position and none when it was applied elsewhere. They therefore conclude (as would seem reasonable from a consideration of normal growth) that normal flexure is brought about by increased growth of the left side, and they suggest that the temperature supplied by

Dareste may have been excessive for the less susceptible tissues of the embryo proper.

It may also perhaps be suggested that the fact of a whole batch of eggs showing heterotaxia may have been due to a genetic symmetry factor, similar to those known to reverse the asymmetry of gasterops (see Driver, Boycott, and Garstang, 1925).

The only other work of a similar nature is that of Vogt (1927), who invented a method whereby one complete half of the egg of a Urodele was cooled and the other half heated with a negligible zone exposed to intermediate temperature. He wanted to discover whether the heated half of the egg alone, with its portion of ' organiser ', would form a whole embryo, and thus test Spemann's theory. He obtained embryos much inhibited in the growth of the cooled half of the body, and these when placed under normal conditions regulated towards the normal condition.

During the present work no case has been found in which anything approaching heterotaxia has been produced either by heating on the right or on the left. Heterotaxia has occurred twice, but in each case the orientation of the embryo in the egg was exactly reversed, the head being in the position where the tail normally develops and was doubtless genetic in origin.

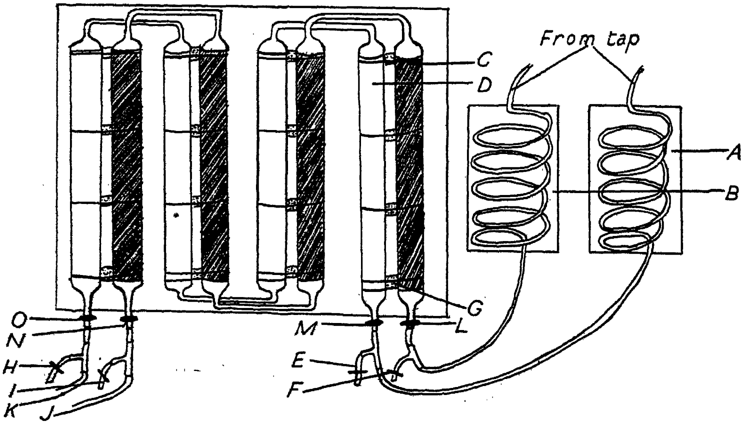
Grateful acknowledgement for suggestions and aid is made to Professor J. S. Huxley under whose direction the work has been carried out.

The work has been carried out with the aid of a grant from the Government Grant Committee of the Royal Society.

APPARATUS.

The apparatus used was devised by Professor J. S. Huxley and constructed by Mr. D. A. Kempson, but various alterations and modifications were introduced from time to time. A photograph of the apparatus is given in Huxley (1927), and a diagram is given in the present paper (Text-fig. 1). A large chick incubator was used, the drawer of which was fitted with two inlet taps at one end and two outlet taps at the other. Two pieces of soft india-rubber tubing (inner tubes of bicycle tyres) were then fitted on to the inlet taps inside the drawer, and were arranged side by side up and down the drawer, so that a series of parallel tubes was produced ; the other end of each tube was then fitted on to an outlet tap which ran to waste. Through these tubes water of different temperatures was passed ; the temperature of the water in each case was kept constant by being passed through a copper spiral tube, which was immersed in a thermo-regulated bath. A constant flow of water was maintained by means of accurately adjustable stopcocks which were fitted on to the taps ; the required flow of water once determined could then be fixed for any length of time, by adjusting the stopcock. The temperatures of the water at both inflow and outflow were taken at regular intervals in each case. The experimental eggs

TEXT-FIG. 1.



A. Low temperature bath ; the copper spiral passes through water kept at a constant temperature. *B*. High temperature bath. *C*. High temperature tube made of inner tube of bicycle tyre. *D*. Low temperature tube. *E* and *F*. Positions from which temperatures of inflows of water were taken in each case. *G*. Pieces of felt used to keep tubes apart. *H* and *I*. Positions from which temperatures of outflows of water were taken in each case. *J* and *K*. Outflow of water, to waste. *L* and *M*. Adjustable taps for inflow of water. *N* and *O*. Adjustable taps for outflow of water.

were placed between the series of parallel tubes in the drawer so that the high temperature tube would rest on one part of the egg and the low temperature tube would lie opposite to it, in the required position. The tubes were insulated from each other by means of small pieces of felt, which were inserted between the tubes at intervals ; the tubes were here wired together to keep them in position. The eggs themselves rested on felt mats which prevented them from rolling about, and kept them in position.

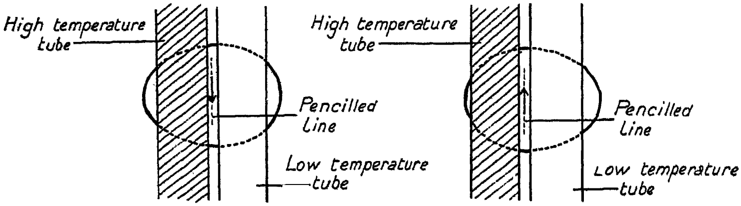
METHOD.

All eggs were obtained from Miss Harrison Bell's farm, and were usually kept in the warm laboratory the night or day before being used for experimental purposes.

Since the orientation of the embryo in the egg is approximately constant, different main regions may be subjected to temperature gradients. Thus the anterior end may be heated and the posterior end cooled (' adjuvant temperature gradient ', Huxley), or the temperature gradient could be reversed (' antagonistic temperature gradient ') ; lateral temperature gradients could also be used, the temperature gradient flowing from right to left or from left to right of an embryo (see Text-fig. 2).

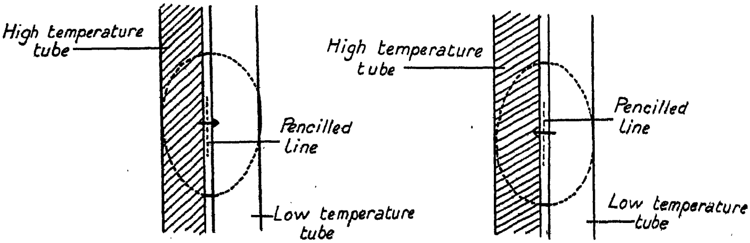
Before the eggs were placed under experimental conditions a pencilled line was drawn on each egg in the required position : this was parallel to the temperature tubes ; in those treated with lateral temperature gradients the line was in the supposed position of the future embryo, but was at right angles to it in those treated with antagonistic and adjuvant gradients. The hot and cold tubes were carefully placed on each side of this line and rested lightly on the egg. The tubes were usually placed from one-quarter to half an inch apart by means of pieces of cork of known thicknesses. In some cases the high temperature tube was placed nearer to the supposed position of the embryo than was the low temperature tube ; this was done in an attempt to obtain greater effects. In those eggs whose embryos were subjected to an antero-posterior or postero-anterior temperature gradient the line was drawn down the long axis of the egg. It was a more difficult task to draw a line in the position of the length of the embryo ; in this case the position of the maximum depth was taken, but here the possibility of error was naturally larger. The pencilled lines were marks of reference ; thus if the eggs were accidentally shifted their former positions could be regained by moving them round until the mark was visible between the tubes once more. This was a necessary precaution, for it proved to be difficult to adjust them exactly in the incubator, since the tubes, being wide, obscured the greater part of the egg ; moreover, if by any chance the eggs were accidentally shifted the temperature gradient might possibly then be oblique relative to the embryo.

TEXT-FIG. 2.



Embryo heated right side, cooled left side.

Embryo cooled right side, heated left side.



Embryo cooled anteriorly, heated posteriorly.

Embryo heated anteriorly, cooled posteriorly.

To illustrate the different positions of the eggs with regard to the hot and cold tubes, when different regions of the embryo were treated.

There were numerous difficulties with regard to the technique of this work, which may be enumerated as follows :

1. A very steep temperature gradient could not be applied, since the normal temperature of incubation of hens' eggs (about 39°·5 C.) is very near to the maximum temperature which they will tolerate. If the temperature used be too high the vitelline membrane is forced up and sticks to the shell, presumably owing to the expansion of the yolk ; this also causes the embryo to be pressed against the shell which usually results in deformities of the embryo. Or perhaps the vitelline membrane adheres to the shell so that when the egg is turned the embryo is prevented from rotating to the top ; this would probably cause the death of the embryo.

2. The orientation of the embryo in the egg is not constant, and the embryo sometimes lies a little obliquely. This may involve a considerable error, especially in those embryos subjected to lateral gradients.

3. If the exact position of the embryo be not successfully estimated when making the pencil line, then the embryo is liable to be covered wholly by either the hot or the cold tube (which would result in a retarded or an advanced condition) and there would be no gradient. On the other hand it was found necessary to have the tubes fairly close together, since a fairly steep gradient is required to induce any effect at all.

4. It was found difficult, if not useless, to keep controls since the experiments were nowhere near the same stage of development to each other. Also the rate of development was found to vary considerably even amongst normals. The best method was therefore found to be to allow those treated with adjuvant temperature gradients to control those treated with the reverse gradient wherever possible. Those treated with lateral right and left temperature gradients were also dealt with similarly wherever possible.

Since the highest temperature possible for use was so near to the temperature at which chicks normally develop, the greater part of the temperature difference was of necessity considerably below the normal temperature of incubation. This causes retardation of the development of the embryo ; to endeavour to compensate for this the temperature of the incubator was raised 1° C.

5. As the beginning of the development of the chick is undergone in the hen, it is not possible to experiment on the very earliest stages of development as in the case of frogs' eggs.

Young embryos were all fixed in Bles's fluid, which was found to stretch the area vasculosa well. Older embryos were fixed with more penetrating agents, Bouin's fluid and acetic bichromate. The stains used were paracarmine for whole mounts and iron haematoxylin for sections.

EXPERIMENTS.

Experiments were started from the earliest possible period. The first two and a half days incubation period was the one most frequently subjected to the temperature gradient, since after that period the whole anterior part of the embryo flexes both laterally and antero-posteriorly. This would result in a changed direction of the gradient relative to the region treated. Thus there was very little scope for experimenting with different periods of growth and differentiation.

Many embryos barely showed any effect of the temperature gradient upon their development, more particularly at the beginning of the work when the gradient was small. Even in the case of those exposed to the greatest possible temperature gradients the temperature difference between the sides or ends of the embryo was probably never more than 4–5° C. at most.

It is not proposed to mention all cases, especially when they merely showed retardation, probably owing to an error in position with regard to the hot and the cold tubes. If they were deformed it was probably due to the same cause in most cases, the hot tube here completely covering the embryo. Therefore, it is proposed to mention only those cases which definitely showed effects of the temperature gradient. However, one example will be given of a definite experiment which will illustrate the great loss of material due to the various causes mentioned above.

Experiment HT.1–HT. 12.

The following abbreviations are used throughout to denote the treatment received by each specimen :

Adj.	Adjuvant temperature gradient.
Antag.	Antagonistic temperature gradient.
Expt.	Period of experimental incubation.
Norm.	Period of normal incubation.
Tubes at M° C. and N° C. respectively.	Average of inflow and outflow temperatures in high temperature tube (M° C.) and low temperature tube (N° C.) respectively.

Since there was just room in the experimental incubator for twelve eggs, this was the number usually taken for each experiment.

Temperatures of thermo-electrically heated baths 20° C. and 50° C. This made the mean temperature of inflow and outflow in the case of the low temperature tube 20° C. (approx.), and 46° C. (approx.) in the case of the high temperature tube.

Six of the eggs were treated with the temperature gradient adjuvant to the embryo and the other six antagonistic to the embryo. These eggs were placed in alternate positions in the incubator, odd numbers being cooled anteriorly and warmed posteriorly.

Of the whole batch only two, HT. 3 and HT. 8, give results of value.

HT. 1 and HT. 4. 74 hours expt. They were both at the primitive streak stage and appeared to be normal, but much retarded. It was assumed that these were cases in which the colder tube almost covered the blastoderm from the beginning. From the point of view of the experiment they were thus worthless.

HT. 2 was infertile.

HT. 6 was at the primitive streak stage but was dead and partially decomposed.

HT. 5 and HT. 7. 74 hours expt.+3 days norm. They were both alive and apparently normal when opened, but HT. 5 was at a stage corresponding to about 50 hours' normal incubation and HT. 7 was at a 60-hour stage (according to Lillie). These, therefore, would seem to correspond to HT. 1 and

HT. 4, and were probably almost wholly retarded when under the experimental conditions.

HT. 10 and HT. 12. 74 hours expt.+3 days norm. They were both dead and also deformed ; both fore- and hind-limbs had developed, but in each case the body was much twisted. Here it appears probable that perhaps owing to the too close proximity of the hot tube the vitelline membrane adhered to the shell, causing the embryo to become fixed and unable to turn when the eggs were turned.

HT. 9. 74 hours antag.+20½ days norm. and allowed to hatch. Now it was in the normal incubator for 20½ days before it hatched, therefore it must have been at a very early stage of development after the 74 hours in the experimental incubator. The chick when hatched proved to be fairly normal, but was weak in the leg and could barely walk. This does not correspond to the treatment it received unless the heat happened to be too severe. However, owing to the great retardation of its development, only the stage corresponding to a normal 12-hour stage could possibly have been subjected to experimental treatment. This 12-hour stage might have been markedly affected since antagonistic temperature gradients often retard the whole development of the chick. On the other hand the general retardation might be due to inhibition by the cold tube alone, in which case the embryo would not have been exposed to a temperature gradient at all. Finally, many normally incubated chicks are often found to be weak in the leg, and this might be the cause of the defect in this case. The proportions of parts appeared to be normal, but numbers of measurements of parts of normal chicks, and, moreover, chicks of the same breed would be necessary for comparison with measurements on experimental chicks before this point could be definitely settled.

HT. 11. The conditions were the same for this case as for HT. 9, but the egg was opened after 17 days in the normal incubator, so that together with the time in the incubator the total time of incubation was twenty-one days. This chick also showed retardation in its time of development, but otherwise appeared normal.

HT. 3 (fig. 1, Pl. 26). The first noteworthy fact about this embryo is its retardation; it roughly corresponds to a stage of development of 22 hours' normal incubation, whereas it was subjected to 74 hours' experimental incubation. As may easily be inferred from the figure, the embryo was subjected to an antagonistic gradient. The egg was opened after 74 hours' experimental treatment.

The most striking feature about the actual blastoderm is the tremendous growth of the area vasculosa posteriorly and its sparseness anteriorly; this is very marked when compared with a normal blastoderm. The neural folds are poorly developed anteriorly for a stage in which there are four pairs of somites.

Professor J. P. Hill, F.R.S., very kindly examined this embryo and made the following approximate measurements in comparison with a normal embryo of a corresponding stage of development in arbitrary micrometer units.

	To Anterior end of the Primitive Knot.	*From Anterior end of Primitive Knot to end of Primitive Streak.*
Normal embryo of four pairs somites	46	30–2
HT. 3. Four pairs somites .	41	40

Thus here the proportion of embryo to primitive streak is considerably modified from about 1·5 to 1·0. The area between the end of the primitive streak and the area vasculosa is relatively even more enlarged.

HT. 8 (fig. 2, Pl. 26). 74 hours adj. The area vasculosa appeared normal anteriorly, but was less extensive than normal elsewhere. The fact that the anterior part of the area vasculosa had not grown abnormally large, though heated, has been observed in other cases, and is probably due to the presence of the proamnion at these stages.

The brain is very well formed, deeper than usual at this stage and well differentiated, the fore-, mid-, and hind-brains being more distinct than normal (see Lillie, p. 99). The trunk flexure is definitely abnormal, being far too great. This may be due to the fact that acceleration of growth of the head region caused excess antero-posterior growth resulting in the greater flexure.

This experiment illustrates the great loss of material possible. However, the experiment described was perhaps more unfortunate than most.

Embryos will now be described which illustrate interesting points and throw light on the subject; naturally it would be useless to describe all embryos, since some are similarly affected, which would result in mere repetition.

HT. 18 (fig. 3, Pl. 26). 72 hours adj.+48 hours norm. Tubes 47° C. and 22° C. The embryo had been dead some time when the egg was opened. It is, however, interesting to note the very short trunk region compared with that of the head, and the poor development of the central nervous system in the trunk relative to that in the head. The neural folds in the trunk are also abnormal, being much convoluted.

HT. 49 (fig. 4, Pl. 26). 72 hours adj. Tubes 46° C. and 28° C. The anterior portion of the area vasculosa, which is usually rather narrow at this stage, appears to be normal; the posterior end, however, is underdeveloped and the sides unusually well developed. The area vasculosa shows an interesting gradation in the development of its blood system. Anteriorly the blood-vessels are well formed and complete, the marginal vein is particularly large and well developed, and blood islands are no longer present; posteriorly blood islands are plentiful and blood-vessels poorly differentiated or absent with the exception of the marginal vein, which, however, is small and poorly developed. This gradation occurs normally but is never so marked. The brain is deep and perhaps somewhat large in comparison with the size of the rest of the body; the flexure of the head, however, appears to be retarded and the eyes are abnormal in position, the choroid fissure pointing outwards instead of downwards. The somites are also retarded posteriorly, for in place of the three to four pairs of somites normally found at this stage posterior to the vitelline arteries, only one pair have developed. In this embryo, there is thus a general retardation of the posterior region and a slight acceleration of the anterior end.

HT. 66 (fig. 5, Pl. 26). 68 hours antag. Tubes 46° C. and

28° C.+7 hours norm. This embryo shows a somewhat premature development of the hind-limb buds. Several embryos were obtained which showed this feature; while those treated with the reverse gradient never showed this development of the hind-limb buds. These differences are perhaps close to the limit of normal variation, though the writer has not observed anything quite so marked. Another figure of this type is shown in Huxley (1927). A curious asymmetry is seen in this embryo, the vitelline artery on the right being larger than on the left, but the left-hand bud being larger than the right one.

Nos. 43 and 48 control each other. No. 43 was cooled anteriorly and heated posteriorly, while No. 48 was treated with the reverse temperature gradient. They were both subjected to experimental incubation for the first 24 hours; the mean temperature of the water in the tubes being 43° C. and 34° C. They were afterwards allowed to develop in a normal incubator for 69 hours. The difference between these embryos is slight, but on comparing them it is possible to see that though No. 48 is at a slightly more advanced stage of development than No. 43, there is a small difference between the relative sizes of heads to hind-limb buds, the head of No. 48 being comparatively greater than that of No. 43. In addition, the relative degree of development of the hind-limb buds is greater in No. 43.

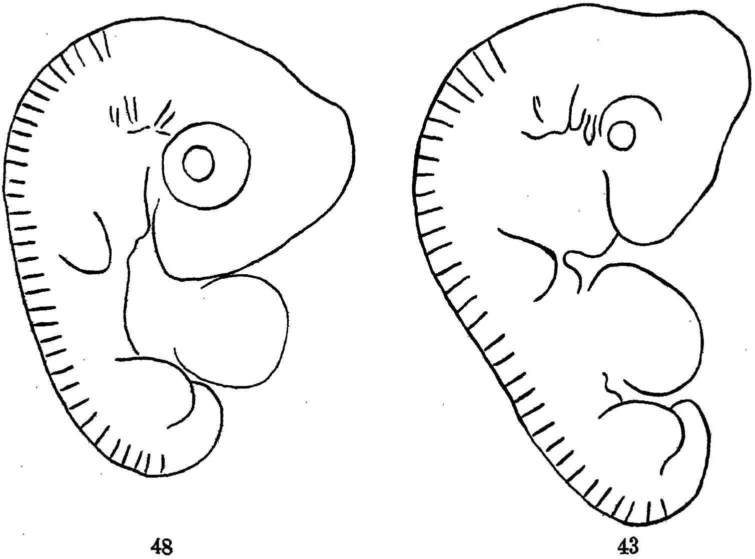
The above specimens are not the only ones obtained by treating embryos to adjuvant and antagonistic temperature gradients, but have been selected because they illustrate effects at different stages of development.

The following are among the interesting specimens obtained by treatment with lateral gradients.

SS. 2 (fig. 6, Pl. 26). 69 hours R–L lat. Tubes 47° and 25° C. The fact that the embryo was heated on the right and cooled on the left is fairly obvious on examination of the blastoderm, the area vasculosa being markedly greater in extent on the heated side. The right anterior horn of mesoblast extends much farther forward than that on the left, and the whole right side of the area vasculosa is much more extensive than the left side. The optic vesicle on the heated side is larger than that on the unheated side ; this point was later verified by measurement in transverse sections of the embryo.

One very remarkable feature is that the somites of each pair are not opposite. There are fourteen pairs of somites, and those on the right—the heated side—are ‘ stepped up ’, starting and ending more anteriorly than those on the left. The somites of the two sides almost exactly alternate, instead of being opposite to each other (fig. 6 a).

TEXT-FIG. 3.



The hind-limbs of 43 are almost at the same stage of development as the hind-limbs of 48, the head of 48, however, is larger than that of 43. (Camera Lucida drawings.)

The thickness of the sides of the neural tube was measured from transverse sections at about every twelfth section by means of an ocular micrometer eye-piece, and it was found strangely enough that the left side—the cooled side—was consistently deeper than the right side. On the other hand, the curvature of the embryo is not what would be expected.

Thickness of Spinal Cord (in arbitrary units).

Right (*heated side*).		*Left* (*cooled side*).	
17·0	0·3	—	16·7
15·0	0·2	—	14·8
13·0	—	1·8	14·8
12·0	—	2·2	14·2
11·0	—	2·0	13·0
13·0	—	—	13·0
11·0	—	2·0	13·0
10·0	—	1·0	11·0
10·0	—	1·0	11·0
9·0	—	2·0	11·0
8·8	—	2·0	10·8
9·0	—	2·0	11·0
9·5	0·5	—	9·0
9·8	—	0·2	10·0
9·8	—	0·2	10·0
10·0	—	0·5	10·5
10·0	—	0·6	10·6
9·9	—	1·3	11·2
11·0	—	1·0	12·0
12·5	—	0·5	13·0
12·2	—	1·6	13·8
14·0	1·0	—	13·0
	2·0	21·9	

Mean difference is in favour of cooled side =0·86.

The sections were also examined to determine whether the thickenings of the neural crest, which later form the spinal ganglia, were opposite to the somites. This was the case so far as could be seen, the thickenings at this stage being only slightly marked.

SS. 1 (fig. 7, Pl. 26). 69 hours. L-R lat.+24 hours norm. Tubes 47° C. and 25° C. The area vasculosa is much more developed on the heated side, but it will be seen that the vitelline arteries and veins are better developed on the cooled side than is the case in SS. 5 (see below). This probably shows that some regulation has occurred during the 24 hours' normal incubation. Here the somites appear to be quite normal in position, and yet it seems obvious that the embryo was accurately heated on its left side owing to the greater development of the area vasculosa on that side. The left fore-limb bud is slightly larger than the right one, but the hind-limb buds are apparently symmetrical. The posterior bud instead of bending in the same direction as the head, which is normal, bends in the opposite direction.

SS. 5 (fig. 8, Pl. 26) and SS. 6 (see Huxley, 1927, fig. 16) were embryos which were treated experimentally for three days and then opened. The temperatures of hot and cold tubes were 47° C. and 25° C. respectively; SS. 5 was heated on its left side and cooled on its right side, and SS. 6 was treated with the reverse temperature gradient.

In each case the area vasculosa and its blood-vessels are very well developed on the heated side, contrasting strongly with those on the cooled side which are very poorly developed. The vitelline arteries and veins show this difference markedly. It it interesting to note that in each case the posterior vitelline vein, which usually runs from the direction of the posterior end of the embryo and then straight to the posterior end of the area vasculosa towards the marginal vein, lies well over towards the heated side of the area vasculosa. The anterior vitelline vein in SS. 5 joins the marginal vein on the right of the blastoderm instead of running in the centre and joining the marginal vein directly anterior. The area vasculosa from the junction of the anterior vitelline vein with the marginal vein towards the left probably corresponds to the left anterior horn of mesoderm which has increased abnormally. The same was noticed in the case of SS. 6. Here again in both cases the somites were 'stepped up' on the heated sides, and in addition the last-formed somites on the heated sides were further differentiated than on the cooled sides (fig. 8a).

The thickness of the spinal cord on each side was measured from transverse sections of SS. 5, and the same result was obtained as in the case of SS. 2, namely that on the whole the spinal cord was thicker on the cooled side.

Here again the thickening of the neural crest seemed to coincide with the somites (see Lehmann, 1927, for further information). It is most unfortunate that the remaining eggs of the experiment when opened all revealed dead and deformed chicks. Apparently three days in the incubator without turning the eggs was too long a period for them to be left, though both SS. 5 and SS. 6 were quite movable and apparently normal and healthy.

THICKNESS OF SPINAL CORD (in arbitrary units).

Right (*cooled side*).		*Left* (*heated side*).	
17·0	—	—	17·0
19·0	1·0	—	18·0
16·0	0·2	—	15·8
17·0	1·0	—	16·0
21·0	1·0	—	20·0
18·0	—	0·5	18·5
18·0	—	2·0	20·0
18·5	0·5	—	18·0
19·0	—	—	19·0
18·0	1·5	—	16·5
17·5	1·2	—	16·3
16·0	—	—	16·0
	6·4	2·5	

Mean difference is in favour of cooled side =0·33.

No. 62 (fig. 9, Pl. 26). 55 hours. R–L lat.+18 hours norm. Tubes 46° C. and 24° C. A number of embryos were treated thus with the same result (see also Huxley, 1927, fig. 15).

As usual the area vasculosa on the heated side is more extensive than on the cooled side. The flexion of the head seems to be somewhat inhibited, and the trunk is bent away from the heated side to the left, so that the prolongation of the axis of the trunk lies on the right side of the head and does not pass through the head as in normal chicks. Several embryos have been obtained in this condition and only when heated on the right side. This condition would appear to be due to the more rapid growth of the embryo on the right side, which would tend to increase the trunk flexure but decrease the head flexure. No other treatment has produced this abnormality, though it is said to occur occasionally amongst incubated chicks (Buchanan, 1926). On the other hand, Dr. Crew of Edinburgh and Dr. Dunn of Michigan have informed Professor J. S. Huxley that they have never seen this abnormality in the very large numbers of chicks which they have opened. The somites were exactly opposite to each other and otherwise normal.

Several specimens from this experiment were examined 2 and 13 days later; all were apparently normal.

No. 3 (fig. 10, Pl. 26). 24 hours norm.+24 hours L–R lat. Tubes 44° C. and 32° C. The very marked abnormal flexure of the trunk might conceivably have been caused in the first instance by a greater growth of the left side due to its more rapid growth caused by extra heat. The embryo when first examined was dead but absolutely free from the egg; the deformity was thus presumably not due to the adherence of the embryo to the shell.

DISCUSSION AND GENERAL RESULTS.

The present work can only be regarded in the light of a preliminary experiment, since it would be necessary to use large numbers of eggs before really detailed conclusions could be drawn.

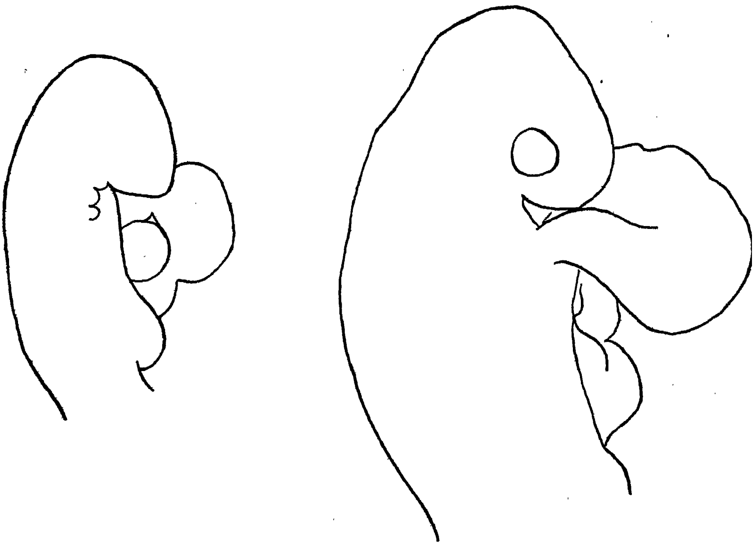
It was found necessary to experiment with the limits of temperature which could be used. A temperature difference of 10° C. between the water in the hot and cold tubes appeared to affect the embryos only very slightly; however, here the actual temperature difference over the embryo itself must be considerably smaller since loss and gain of heat occurs, the shell and albumen being poor conductors of heat. That there was a fall in temperature even through the wall of the rubber tubing was demonstrated by the fact that a thermometer placed between the egg and the high temperature rubber tube registered a lower temperature than that of the water flowing through the tube, and vice versa. In the case of the hot tubes the difference was 1·5° C., in the cold tube only about 1° C.

Since this temperature difference of 10° C. had such small effect on the embryos, greater temperature differences were tried; those most successful were about 26° (from 20° C. to 46° C.) or 22° (from 25° C. to 47° C.). Now 47° C. is a higher temperature than chick embryos can normally tolerate, Jenkinson stating 43° C. to be the maximum temperature under which chicks can develop normally. However, owing to the simultaneous presence of the low temperature tube, the full effects of the hot tube must have been counteracted in most regions.

In almost every case the part of the blastoderm most affected was the area vasculosa, the higher temperature causing an acceleration of growth and development, the lower temperature a retardation. Markedly greater growth of the area vasculosa is seen for example on the right sides of SS. 2 (fig. 6, Pl. 26) and 62 (fig. 9, Pl. 26), on the left side of SS. 5 (fig. 8, Pl. 26), and very markedly at the posterior end of HT. 3 (fig. 1, Pl. 26). These examples are merely a few of the many cases obtained which show that the area vasculosa is very easily affected by heat. In only two cases has the area vasculosa been more extensive in a supposedly unheated part of the blastoderm. In the case of HT. 58, which was actually subjected to a gradient adjuvant for a normally situated embryo, the embryo appears to have been heated on the left, for the area vasculosa on that side is larger than on the right and the somites are also 'stepped up' on the left; thus in this case it is possible that the embryo was situated at right angles to its normal position (according to Duval 2 embryos out of 166 eggs observed were orientated thus). The development of the blood system of the area vasculosa is also markedly affected; in HT. 49 (adjuvant temperature gradient) the marginal vein is much wider anteriorly than is usual, while at the posterior end it is insignificant in size. Here the distinct gradation of the development of the blood system is notable. The different arrangement of blood-vessels occurring in those treated with a lateral temperature gradient is worthy of note; in SS. 5 (heated left) the anterior vitelline vein is displaced to the right: exactly the reverse conditions were observed in SS. 6, heated right side and cooled left, when the anterior vitelline vein was markedly displaced to the left.

In many cases a distinct alteration of the proportion of parts of the embryo is apparent. In HT. 3 (antagonistic temperature gradient) the primitive streak is long in comparison with the stage of development of the embryo, the head region is small and under-developed for the number of somites present. Here rates of growth have been affected. The opposite effects are seen in HT. 8 (fig. 2, Pl. 26) (adjuvant gradient), where the head is deep and well formed but the rudiments of the hind-limb buds are not yet visible; this embryo could only be compared with HT. 66 (fig. 5, Pl. 26) (antagonistic temperature gradient), which is at approximately the same stage of development but possesses well-marked hind-limb buds though a relatively smaller head. In others, No. 43 (Text-fig. 3) (antagonistic temperature gradient) and No. 48 (Text-fig. 3) (adjuvant temperature gradient), the relative development of head- and fore-limb buds to hind-limb buds differs according to treatment. The difference here, however, is slight and possibly within the range of normal variation.

TEXT-FIG. 4.



Embryos showing enlargement of the heart due to heating. (SS. 24) (*a*) Embryo heated right side and cooled left for about 3 days. (HT. 51) (*b*) Embryo heated anteriorly and cooled posteriorly for about 4 days.

Other regions whose growth have been affected by temperature in these experiments are (1) the heart (Text-fig. 4, *a* and *b*), which in one case was very much enlarged when heat was applied on the right (SS. 24) (Text-fig. 4, *a*), and in another when heated anteriorly (Text-fig. 4, *b*); (2) the optic vesicles, e.g. SS. 2

(fig. 6, Pl. 26) heated on the right, in which the right optic vesicle is larger than the left.

One very interesting result obtained in embryos laterally heated was the displacement of the somites, those on the heated side being often shifted to positions anterior to those on the cooled side. This forward shifting of the somites was only found to occur in embryos subjected to lateral temperature gradients but did not by any means occur in every case so treated. This condition of the somites was also only found in those embryos which were examined directly after experimental treatment, that is, before they were ever normally incubated. SS. 2 shows this condition of the somites very clearly, the somites in the middle almost alternating with each other. Now SS. 1 belonged to the same batch as SS. 2 and was thus treated for the same length of time experimentally, but the embryo was heated left and cooled right, and in addition subjected to 24 hours' normal incubation. SS. 1, however, does not show this abnormal arrangement of the somites in any degree whatever, and yet the embryo was apparently accurately treated, for the blood system of the area vasculosa and its size and shape show marked indications of the effect of the temperature gradient having been in the correct position. It may perhaps be possible that in this case the somites had regulated back to their normal position. Or else the precise position of the gradient with reference to the midline of the embryo is decisive. Another set of embryos, which were heated right and cooled left for a considerable time and with the greatest possible temperature gradient, did not exhibit this 'stepped up' appearance of the somites on the heated side; unfortunately, in this case, not one embryo was examined directly after the period of experimental incubation, but the majority after a further 18 hours' normal incubation (61 and 62). However, this period of 18 hours' normal incubation might have proved long enough for the regulation of the somites to have taken place. This point, however, can merely be put forward as a tentative suggestion, the number of cases obtained being insufficient to allow of a definite conclusion.

The problem of the forward shift of the somites on the heated side is difficult to solve. If the view be adopted that the heated side grows more rapidly, so causing the forward displacement of the heated side, then it would be expected that organs, such as the eyes and ears, would also be displaced. So far the evidence does not appear to indicate that such is the case. In the cases so far studied, however, the thickenings of the neural crest are still apparently opposite to their respective somites ; it therefore appears that the arrangement of the ganglia are determined by the position of the somites ; this bears out Lehmann's work (1927). Kopsch (1927) produced chick embryos in which, after localized injuries produced by the electric cautery, the members of pairs of somites were not opposite to each other but sometimes even a whole somite out. Here the effect produced was probably purely mechanical in nature, and does not bear upon our problem. In the embryo of the rabbit it has been observed that the somites of a pair are frequently not exactly opposite to each other, but in some cases as much as half a somite out. This, however, never appears to happen in the normal chick.

The investigation of the powers of regulation of the chick embryo after treatment was very difficult owing to the fact that it was impossible to ascertain the effect which the temperature gradient had had on the embryos without opening the eggs after treatment. Nevertheless, no living embryos were obtained which showed any appreciable signs of a disproportion of parts, or side-to-side asymmetries after further normal treatment for several days. This bears out the experiments of Vögt (1927) who affected Urodele embryos markedly by differential temperatures. When placed under normal conditions, however, they regulated very rapidly.

The area vasculosa was the most markedly affected region of the embryo, and many embryos were obtained which showed only asymmetries of the area vasculosa. This is to be expected as its area is not restricted ; however, it is very easily regulated back to the normal condition.

It thus appears that statistical methods in which large numbers of eggs are used would be necessary for the complete study of the degree of regulation of the embryos after treatment. Since, however, definite positive effects have already been obtained upon growth and differentiation of extra-embryonic regions, and the arrangement of their blood-vessels, upon proportions of parts of the embryo, flexure of the embryo, and the arrangement of the somites of the two sides, it is evident that the method may have its uses in a study of developmental physiology.

Tabular Summary of Experiments.

Eggs infertile or undeveloped	53
Deformed specimens	33
Retarded but otherwise normal	28
Apparently unaffected when opened immediately after treatment	12
Later stages of all ages, which, after experimental incubation, have been subjected to varying periods of normal incubation and are normal in appearance, perhaps owing to regulation	25
Cases showing a definite effect of the temperature gradient . .	35
Total number of eggs used	184

Summary.

1. Owing to the difficulty of ascertaining the exact position of the embryo in the egg there was much waste of material. Hens' eggs are not ideal for temperature gradient experiments, for the mean between the temperatures which can be used is below normal incubation temperature. The egg of a cold-blooded animal would be far simpler to deal with.

2. The part most easily affected by a temperature gradient was the area vasculosa and its blood-vessels. This was to be expected, since the size of this extra-embryonic part is not strictly limited; the arrangement of the blood system of the area vasculosa was also modified in some cases.

3. Slight disproportion of parts was effected in some cases. The head was sometimes slightly more developed than the posterior end, and in some cases the posterior limbs were precociously developed. Differences in size between the limb buds on each side also occurred. The ratio of embryo to primitive streak was decreased considerably in the case of two embryos, treated with antagonistic gradients.

TABLE I. THE EFFECTS PRODUCED BY ANTAGONISTIC AND ADJUVANT TEMPERATURE GRADIENTS TABULATED TO SHOW THE APPROXIMATE NUMBER OF TIMES DIFFERENT EFFECTS HAVE BEEN PRODUCED.

		Heated Part of Area Vasculosa Enlarged.	*Blood-vessels of Heated Side Enlarged.*	*Primitive Streak Elongated.*	*Heart Enlarged.*	*Head Enlarged.*	*Hind-limbs Prematurely Developed.*
HT. 3.	Heated post.	Markedly	—	Markedly	—	—	—
HT. 5.	” post.	—	—	—	—	—	Very slightly
HT. 8.	” ant.	—	—	—	—	Slightly	—
HT. 18.	” ant.	—	—	—	—	”	—
HT. 49.	” ant.	—	Markedly	—	—	—	—
HT. 51.	” ant.	—	—	—	Very much	—	—
HT. 66.	” post.	—	—	—	—	—	Slightly
HT. 68.	” post.	—	—	—	—	—	”
HT. 83.	” post.	Markedly	—	Fairly	—	—	—
43.	” post.	—	—	—	—	—	Very slightly
47.	” post.	—	—	—	—	—	”
48.	” ant.	—	—	—	—	Very slightly	—

TABLE II. THE EFFECTS PRODUCED BY LATERAL TEMPERATURE GRADIENTS.

		Heated Side of Area Vasculosa Enlarged.	*Somites Higher on Heated Side.*	*Limbs Larger on Heated Side.*	*Heart Enlarged.*	*Blood-vessels of Heated Side Enlarged.*	*Trunk Flexure too Great, Head Flexure too Small.*
SS. 1.	Heated left	Fairly	—	Slightly	—	Appreciably	—
SS. 2.	„ right	Much enlarged	Almost alternate	—	—	—	—
SS. 5.	„ left	Much	Markedly	—	—	Markedly	—
SS. 6.	„ right	„	„	—	—	„	—
SS. 19.	„ left	—	—	Slightly	—	—	—
SS. 20.	„ right	Fairly	—	—	—	—	—
SS. 24.	„ right	„	Appreciably	Fairly	Considerably	—	—
3.	„ left	Slightly	—	—	—	—	—
4.	„ right	„	—	—	—	Slightly	—
6.	„ left	„	Slightly	—	—	—	—
8.	„ right	„	—	—	—	—	—
13.	„ right	„	—	—	—	—	—
55.	„ right	—	—	—	—	Slightly	—
57.	„ right	Slightly	—	—	—	—	—
61.	„ right	Fairly	—	—	—	—	Appreciably
62.	„ right	„	—	—	—	—	„
63.	„ right	Markedly	—	—	—	—	„
66.	„ right	„	—	—	—	—	—
70.	„ right	Slightly	—	—	—	—	Slightly
78.	„ right	„	—	—	—	Slightly	—
81.	„ right	Fairly enlarged	—	—	—	—	Fairly

4. In some embryos treated with a lateral temperature gradient the somites had become shifted up on the heated side. The greatest effect was obtained in an embryo whose somites of one side alternated with those of the opposite side. It is possible that this condition may be regulated after normal incubation ; results, however, were too few for certainty.

5. The numbers used were not sufficient for the conclusive determination of the degree of regulation which experimentally treated embryos underwent. However, all late stages when examined were normal; whether these cases were correctly treated or not it is impossible to say.

LITERATURE CITED.

Bellamy, A. W. (1919).—" Differential susceptibility as a basis for modification and control of early development in the frog ", ' Biol. Bull.', 37.

Bellamy and Child (1924).—" Susceptibility in Amphibian development ", ' Proc. Roy. Soc. Lond.' (B), 94.

Buchanan, W. J. (1926).—" Regional differences in rate of oxidations in the chick blastoderm as shown by susceptibility to hydrocyanic acid ", ' Journ. Exp. Zool.', 45.

Cannon, H. G. (1923).—" On the metabolic gradient of the frog's egg ", ' Proc. Roy. Soc.' (B), 94.

Copenhaver, W. M. (1926).—" Experiments on the development of the heart of Amblystoma ", ' Journ. Exp. Zool.', 43.

Dareste, C. (1877).—' Recherches sur la production artificielle des monstruosités.' Paris, 1877.

Diver, C., Boycott, A. E., and Garstang, S. (1925).—" The inheritance of inverse symmetry in Limnaea peregra ", ' Journ. Genetics ', 15.

Ekman (1925).—" Experimentelle Beiträge zur Herzentwicklung der Amphibien ", ' Arch. Ent.-Mech.', 106.

Geinitz, B. (1925 *b*).—" Zur weiteren Analyse des Organisationszentrums ", ' Zeit. Ind. Abst. u. Vererb.', 37.

Harrison (1918).—" Experiments on the development of the fore-limb of Amblystoma, a self-differentiating equipotential system ", ' Journ. Exp. Zool.', 25.

—— (1921).—" A relation of symmetry in transplanted limbs ", ibid., 32.

—— (1925).—" The effects of reserving the mediolateral or transverse axis of the fore-limb bud in the Salamander embryo (Amblystoma punctatum) ", ' Arch. Ent.-Mech.', 106.

Huxley, J. S. (1927).—“ The modification of development by means of temperature gradients ”, ibid., 112.

Hyman, L. H. (1927).—“ The metabolic gradients of vertebrate embryos. III. The chick ”, ‘ Biol. Bull.’, 52.

Kopsch, Fr. (1927).—“ Primitivstreifen und organbildende Keimbezirke beim Hühnchen untersucht mittels elektrolytischer Marken am vital gefärbten Keim ”, ‘ Zeit. Mikros. Anat. Forsch.’, 8.

Lehmann, Fritz E.—“ Further studies on the morphogenetic role of the somites in the development of the nervous system of Amphibians ”, ‘ Journ. Exp. Zool.’, 49.

Shaw, Dean, and Tazelaar (1927).—“ The effect of a temperature gradient on the early development of the frog ”, ‘ Brit. Journ. Exp. Biol.’ (in press).

Stöhr, P. (1925).—“ Experimentelle Studien an embryonalen Amphibienherzen ”, ‘ Arch. Ent.-Mech.’, 106.

Vogt, Walther (1927).—“ Über Hemmung der Formbildung an einer Hälfte des Keimes ”, ‘ Anat. Anzeiger ’, 63.

Warynski, St., et Fol, H. (1884).—“ Recherches expérimentales sur la cause de quelques monstruosités simples et de divers processus embryogéniques ”, ‘ Recueil Zool. Suisse ’, 1.

DESCRIPTION OF PLATE 26.

Fig. 1 (HT. 3). 74 hours’ antagonistic temperature gradient. Temperatures of tubes 46° C. and 20° C. Note the enormous growth of the area vasculosa posteriorly and its sparseness anteriorly, also the abnormal length of the primitive streak.

Fig. 2 (HT. 8). 74 hours’ adjuvant temperature gradient. Temperatures of tubes 46° C. and 20° C. Note relatively large size of head, the abnormal trunk flexure and the posterior end which is somewhat retarded.

Fig. 3 (HT. 18).—72 hours’ adjuvant temperature gradient. Temperatures of tubes 47° C. and 22° C. Note size of head compared with that of the body.

Fig. 4 (HT. 49).—72 hours’ adjuvant gradient, tubes 46° C. and 28° C. Note width of marginal vein anteriorly and compare it with the posterior portion. Blood islands still plentiful posteriorly, somites not formed below the vitelline arteries.

Fig. 5 (HT. 66).—68 hours’ antagonistic gradient, tubes 47° C. and 25° C. Note somewhat premature development of the hind-limb buds.

Fig. 6 (SS. 2).—69 hours’ right to left temperature gradient, tubes 47° C. and 25° C. Note the area vasculosa is considerably enlarged on the right, the heated side, the optic vesicle is a little larger on the right, and the somites on the heated side appear to be ‘ stepped up ’ so that they almost alternate.

Fig. 6 a.—Portion of fig. 6 on a larger scale, showing the somites.

Fig. 7 (SS. 1). 69 hours' left to right temperature gradient plus 24 hours' normal incubation, tubes 47° C. and 25° C. Note the left side of the area vasculosa is larger than the right side, this is not very apparent in the figure, the blastoderm having been damaged; the left fore- and hind-limb buds are also slightly larger than those on the right.

Fig. 7 a.—This figure represents merely the embryo of the preceding figure enlarged to show that the somites are normal in position, not being 'stepped up'. Left fore-limb larger than right, left hind-limb tilted so as to appear smaller than right, but actually is slightly larger.

Fig. 8 (SS. 5).—72 hours' left to right gradient, tubes 47° C. and 25° C. Note the much greater size of the area vasculosa on the left than on the right and the difference between the sizes of the blood-vessels supplying the respective sides of the area vasculosa. Here again the somites are 'stepped up' on the heated side.

Fig. 8 a.—Portion of fig. 8 on a larger scale, showing the somites.

Fig. 9 (62).—55 hours' right to left gradient, tubes 46° C. and 24° C., plus 18 hours' normal incubation. Area vasculosa on right markedly larger than on left. Note also the abnormal curvature of the embryo, the axis of the posterior part of the trunk passes to the right of the head where normally it passes through the head.

Fig. 10 (3).—24 hours' normal incubation, then a lateral left to right temperature gradient for 24 hours; tubes 44° C. and 32° C. Marked abnormal trunk flexure might possibly have been due to greater growth of that side caused by the heat.

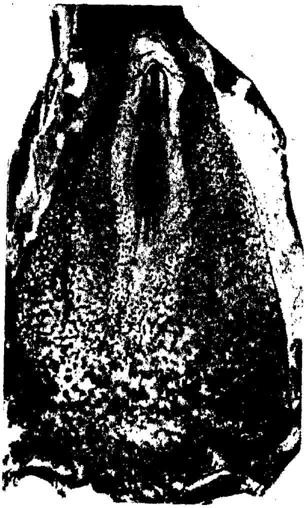


Fig. 1 (HT. 3).

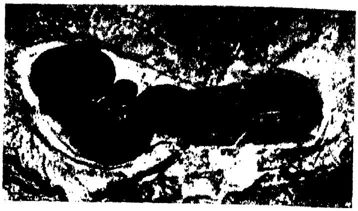


Fig. 2 (HT. 8).

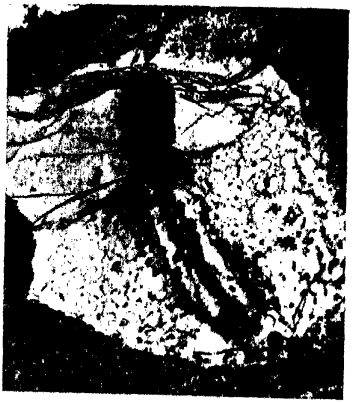


Fig. 3 (HT. 18).

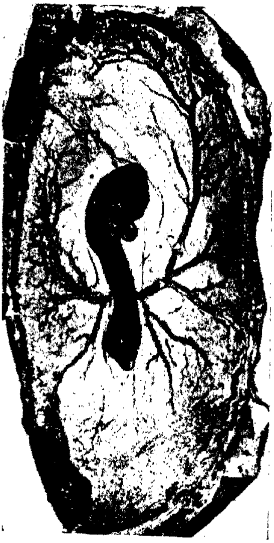


Fig. 5 (HT. 66).

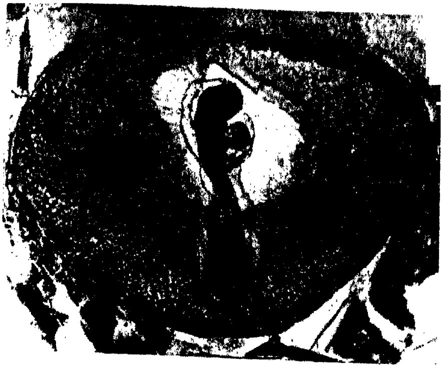


Fig. 4 (HT. 49).

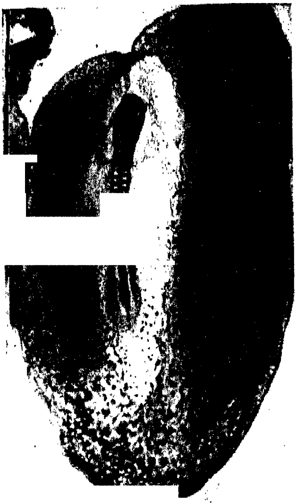


Fig. 6 (SS.2).

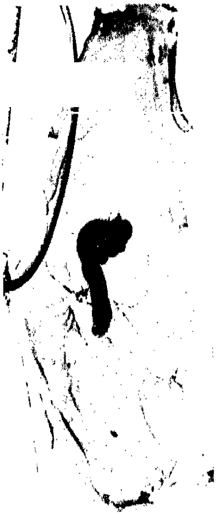


Fig. 7 (SS.1).

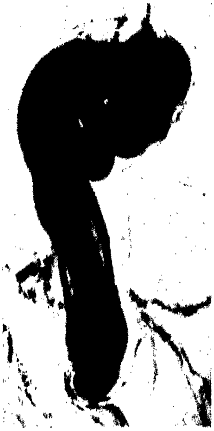


Fig. 7 a (SS.1).

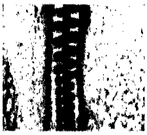


Fig. 6 a.

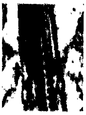


Fig. 8 a.

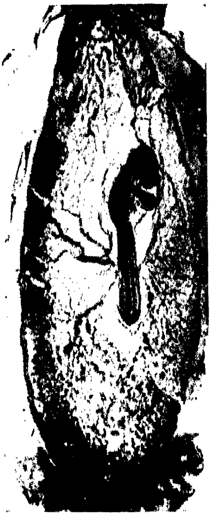


Fig. 8 (SS.5).

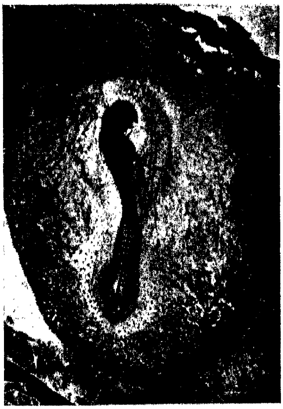


Fig. 9 (62).

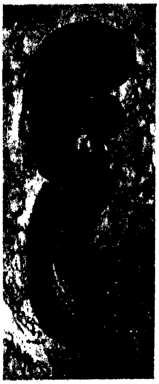


Fig. 10 (3).

The Morphology and Development of the Genitalia and Genital Ducts of Homoptera and Zygoptera as shown in the Life Histories of Philaenus and Agrion.

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With Plates 27, 28, 29.

PART I. GENITALIA.

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INTRODUCTION AND HISTORICAL SURVEY.

DURING the latter half of the last century two schools of thought existed among entomologists regarding the origin of the gonapophyses of insects ; while Verhoeff, Weismann, Huxley, Cholodkovsky, Dewitz, Kraepelin, and others believed in the appendage origin of these structures, Lacaze-Duthiers, Haase, Grassi, and others were of opinion that they were only integumental outgrowths and had nothing to do with ambulatory legs.

The work of Wheeler (1893) on Xiphidium showed conclusively that the embryonic appendages of the genital segments of the abdomen directly developed into the gonapophyses. This new piece of evidence threw considerable light on the first view, though Heymons (1896), while reviewing the work of Wheeler, pointed out that it might be due to the early appearance of the gonapophyses in the embryo of Xiphidium that Wheeler was unable to detect the entire discontinuity of the embryonic appendages and the rudiments of the gonapophyses. As Heymons's criticism was not sufficiently supported by facts, Wheeler's view has remained unchallenged ever since, and there is general agreement among entomologists regarding the appendage nature of the genitalia.

Dewitz (1874) showed that in the female nymph of Locusta (Phasgoneura) viridissima the primitive pair of buds of the ninth segment split into two to give rise to an inner and an outer pair. The inner pair were therefore considered by many entomologists to be the homologues of the endopodites and the outer pair to be the exopodites of the biramous crustacean limb. The anterior ovipositor lobes (those on the eighth abdominal segment), on account of their median ventral position, similar to that of the inner ovipositor lobes on the ninth, were regarded as endopodites of that segment, the exopodites having been lost (Escherich, Walker, Verhoeff).

Verson and Bisson (1896) showed that the aedeagus, the single tubular intromittent organ in the adult Lepidopterous male, could be traced to a pair of appendages in the earlier stages. They were followed by Michealis (1900), who got almost similar results in the case of Hymenoptera. Zander (1900, 1901, 1904–5) showed that the origin of the aedeagus is identical in Hymenoptera, Trichoptera, and Lepidoptera. Palmen (1884) had already brought to the notice of entomologists the paired penis condition of Ephemeroptera.. Escherich (1903) and Verhoeff (1910) pointed out the double condition of the penis in Thysanura. Thus the penis (aedeagus) was considered by many entomologists as representing the original endopodites. Escherich and Walker (1919) compared the ' parameres ', a pair of outgrowths present on either side of the aedeagus, to the endopodites, leaving the homology of the aedeagus out of consideration. Walker, when he states that the penis in the different groups of Orthopteroid insects may not be homologous, implies that, in the groups in which ' parameres ' are present, the penis may have a different origin and may not represent the endopodites.

Kershaw and Muir (1922), faced with the difficulty of homologizing three pairs of appendages on the ninth segment in the Homopterous male, assigned the anterior pair (the sub-genital plates) to the preceding segment and regarded them as homologues of the anterior ovipositor lobes.

Crampton (1922) from his studies on the Homopteron genitalia arrived at the conclusion that the ' hypandrium ' or ' hypopygium ', the sternite beneath the genital apparatus of the male, might occur as a distinct plate or might be indistinguishably united with the pleural region of the ninth uromere. His view, as summarized by Muir, is ' that the line of evolution of the Hypandrium ' is from fixed processes to large articulated processes. In other words the sub-genital plates of the ninth segment are nothing more than the expansions of the hypandrium.

Singh-Pruthi (1924) traced the development of the male genitalia in the Jassid Idiocerus and found in the nymphs the two pairs of appendages of the ninth segment become three by the splitting of the inner pair. The inner pair produced by the splitting of the original inner pair fuse to form the aedeagus and the outer develop into ' parameres '. He therefore argued that neither the penis nor the parameres alone could be homologized to the inner ovipositor lobes of the female, but the two together represented the endopodites. He criticized the views of Kershaw and Muir and maintained that the sub-genital plates were coxites of the ninth segment.

Muir (1926), replying to Singh-Pruthi's criticisms, questioned his conclusions in several respects. In the following pages I have endeavoured to re-examine the whole question in the light of Muir's criticism of Singh-Pruthi's work from an ontogenetic study of the genitalia in the Cercopid Philaenus leucophthalmus L. Moreover, I wish to include in this paper a few notes on the development of genitalia of the Zygopterid dragon-fly Agrion.

MATERIAL AND METHOD.

The material for this work (specimens of Philaenus and Agrion) was obtained by collecting locally. Philaenus leucophthalmus L. is the same species on which Kershaw and Muir based their conclusions. For purposes of comparison I have also examined specimens of the sugar-cane-frog-hopper Perkinsiella saccharicida received from the entomologist in charge of the Sugar-cane Pest Investigation Station, Trinidad. Carl's aceto-formol was found to be a very good fixative. The specimens were fixed in cold for twenty-four hours and washed twice in 70 per cent. alcohol. The early stages were embedded in paraffin, but, for the later ones, the celloidin method as recommended by Tillyard was followed. Ehrlich's acid haematoxylin, with or without eosin and Mallory's triple stain, were very generally found to give good results. Woodland's picro-indigo carmine was also used in some cases.

PHILAENUS LEUCOPHTHALMUS, L.

Male.

The ninth abdominal segment is well marked and is characterized by the well-developed leaf-like appendages, the sub-genital plates. The two sub-genital plates, where they meet at their bases, leave but a narrow strip of the sternum anteriorly. Dorsal to the sub-genital plates are situated a pair of well-chitinized processes, 'the parameres', on either side of the median aedeagus. The aedeagus bears, in addition to the 'parameres', a pair of processes at its tip, and these form the two backwardly curved aedeagal hooks. The tenth abdominal segment is easily recognized by the presence of the characteristic 'surgonopods'; fig. 1, Pl. 27, gives a ventral view of the last two abdominal segments.

Female.

The eighth abdominal segment is shorter than the ninth. The 'anterior' ovipositor lobes are borne by the eighth. The 'basivalvulae' of the anterior ovipositor lobes represent practically the whole of the visible sternum of the eighth segment. The ninth sternum bears two pairs of appendages, an 'outer' and an 'inner'. The outer are more flattened and are free, whereas the inner are fused with one another on their dorsal margins, forming a somewhat inverted boat-shaped structure. The 'anterior' and the 'inner' lobes are so interlocked that they enclose a cavity between them. This interlocking is established by means of a pair of small ridges of the inner lobes fitting into a pair of grooves of the anterior lobes. Fig. 2, Pl. 27, gives a ventral view of the last three segments of the abdomen of the female. A cross-section of the ovipositor is given in fig. 3, Pl. 27. A median sclerite situated between the inner lobes connects the two lobes at their bases. The opening of the median accessory gland is situated above this sclerite. The anterior lobes fuse at their bases, and at that region the bases of the inner lobes project forward between the body-wall and the fused anterior lobes. The vaginal opening is situated above the fused bases of the anterior lobes.

Development in the Male.

In the earliest nymphal stages there is a longitudinal groove running along the mid-ventral line of the ninth sternum (vide fig. 4, Pl. 27). The ectodermal cells that form the grooves are much bigger than those of the remaining ectoderm. The gonapophyses develop from the sides of this groove.

In the late first instar the rudiments of the appendages are better developed. Fig. 5, Pl. 27, shows a cross-section of the late first instar. The rudiments of the sub-genital plates are well marked. The inner pair, 'the penis lobes' (the paramere lobes of Singh-Pruthi), are also indicated.

During the third nymphal stage the penis lobes, while giving rise to the parameres below, by the fusion of their lips develop into a single median aedeagus. The groove between the penis lobes thus becomes the tube of the intromittent organ, and this tube in the adult serves to extend the ejaculatory duct to the orifice. The fusion of the penis lobes takes place from behind forwards. Fig. 6, Pl. 27, shows the aedeagus rudiments still remaining as a groove, whereas in fig. 7, Pl. 27, the two lips have fused. The parameres merge into the aedeagus rudiments at their bases.

As development proceeds a pair of ridges are developed along the ventral side of the aedeagus, and as the organ grows out these ridges become so developed as to occupy a somewhat dorsal position at its base (vide fig. 8, r aed. Pl. 27).

During the fourth instar the aedeagal hooks become differentiated at the tip of the intromittent organ.

Development in the Female.

The first instar nymph shows a groove similar to the one noted in the case of the male on the ninth sternum. This groove is bounded on either side by the rudiments of a pair of appendages (vide fig. 9, Pl. 27). A similar groove is situated on the eighth segment and a pair of rudiments of appendages are also seen on either side of the groove. Towards the close of the first instar each of the appendages of the ninth sternum divides into two. Thus as the nymph grows older two pairs of distinct appendages are seen on segment 9, the inner ovipositor lobes and the lateral ones (vide fig. 10, Pl. 28).

The appendages of the eighth segment also show a similar bifurcation during the second instar. As the nymph grows older the two inner lobes grow markedly, whereas the outer remain stunted. The inner two represent the rudiments of the anterior ovipositor lobes, and the outer the rudiments of the ‘ basivalvulae ’ (vide fig. 11, Pl. 28).

At about the late third instar, the dorsal edges of the inner lobes on the ninth segment become deflected mesially, meet, and then fuse to form the inverted boat-shaped structure already referred to.

During the last instar the anterior and the inner ovipositor lobes get interlocked by a ridge and groove arrangement. A deep incision in the meso-dorsal wall of each anterior ovipositor lobe develops in such a way that an arm-like portion becomes differentiated from the rest of each lobe. In the adult these lobes are probably meant to keep a firm grip on the aedeagus during copulation (vide fig. 3, faol Pl. 27).

AGRION.

Male.

There is only a single pair of appendages in the male on the ninth sternum. The copulatory function having been transferred to secondary structures on the second and third abdominal segments, the inner pair of buds on the ninth sternum are suppressed. The male gonopore is situated in the middle of the ninth sternum between the bases of the appendages (vide fig. 12, Pl. 28).

Female.

A pair of processes are borne on the posterior region of the eighth sternum, and two pairs, an outer and an inner, on the ninth sternum. The outer pair on the ninth is more flattened and each one bears a stylus at its apex. The anterior pair on the eighth and the inner pair on the ninth are interlocked and enclose between them the canal of the ovipositor. The two lobes of the anterior pair are also interlocked. The interlocking is affected by a ridge and groove arrangement. The canal of the ovipositor in the Cercopid Philaenus is open on the ventral side, but in Agrion it is completely closed on the ventral side (vide fig. 13, Pl. 28, which shows a lateral view of the female genitalia and fig. 14, Pl. 28, which gives a cross-section of the ovipositor).

Development in the Male.

In the early male nymph the sternum of the ninth segment bears two processes, each having a stylus at the tip. These processes develop into the coxites of the adults. While examining the cross-sections of nymphs, in one case I noticed a pair of tubercles situated between the rudiments of the coxites on the ninth sternum. These tubercles probably represent the inner pair of buds which normally do not make their appearances.

Development in the Female.

In the earliest nymphal stage the ninth segment bears two pairs of processes, an outer and an inner. Each of the two outer processes bears a stylus at its apex, whereas the inner two are devoid of them. The bases of these two pairs run into each other in such a way as to suggest a common origin. An anterior pair of appendages borne by the eighth sternum is also present as rudiments. During the successive nymphal stages these appendages grow and become more and more chitinized. At the last nymphal stage each of the anterior lobes develops a proliferation of cells on its inner face, and ultimately this results in the formation of a pair of processes (vide figs. 15, 16, and 17, Pl. 28).

DISCUSSION.

The development of the genitalia in the Homopteron Philaenus leucophthalmus L. is of greater importance than that of the Zygopteron Agrion, with regard to the establishment of the fundamental homology of the gonopophyses of the two sexes because of the suppression of the inner pair in the male of the latter insect. Summarizing the development of the genitalia in the Cercopid, in the early stages an inner pair of structures arises from a single pair of buds on the ninth sternum in both the sexes. Such a process has been noted by Dewitz in the female Locustidae, and also by subsequent workers in the male in Hymenoptera and Lepidoptera. So far the development proceeds on identical lines in the two sexes. Later on the inner pair develop into the inner ovipositor lobes in the female, whereas the inner pair of the male undergo complicated changes. The meso-dorsal region of the inner pair in the male differentiates into a pair of processes which unite to form the aedeagus, and the ventro-lateral region develops into the ‘ parameres ’.

The above-described mode of development in the male is in all essentials similar to what Singh-Pruthi found in the Jassid Idiocerus, but he did not go into the development of the female and so could not notice the underlying similarity of development in the earlier stages in the two sexes. He termed the inner pair in the male ' paramere lobes ', but I have preferred to refer to them as ' penis lobes ' on account of the morphological significance of the aedeagus which arises from these lobes. Moreover Singh-Pruthi does not seem to have followed the development of the parameres in detail, for he regarded the division of the ' paramere lobes ' into ' parameres ' and aedeagus lobes as complete. That this is not the case I have shown above, and it is also clear from his fig. 3 *c*, Pl. 5, ' Quart. Journ. Micr. Sci.', 1924. From his study of the comparative morphology of genitalia in the Homoptera, Singh-Pruthi seems to have formed the opinion that the parameres are distinct entities in themselves, as in chitin preparations of adult insects they appear as a pair of separate processes of the ninth sternum. Influenced by this opinion, he failed to notice the details of development in his ontogenetic study and so formed the following conclusion. 'As in Homoptera, in insects in general there are, besides the aedeagus, two pairs of appendages in connexion with the ninth (coxites and parameres), and not one as hitherto believed.' It is rather curious that, while knowing that in some Ephemeroptera such as Callibaetis and Heptagenia, each penis is composed of two parts, an outer and an inner (penis and paramere), and in Trichoptera the parameres are nothing more than the appendages of the aedeagus, Singh-Pruthi should have arrived at the conclusion referred to. I regard the 'parameres' not as distinct entities but as processes of the aedeagus, and as such the aedeagus is the representative of the endopodites of the ninth segment. 'Parameres', as they are present in primitive groups, perhaps originated as processes on the aedeagus to serve as claspers, and they are regarded as such by many authors. They were present very early in phylogeny and it is quite probable that in some groups they have disappeared, e.g. there is some evidence that this has occurred in some of the Culicids.

The question has been raised why the aedeagus, the supposed homologue of the endopodites, develops processes while the homologue in the female remains simple. But there are cases where in the female processes develop on the anterior ovipositor lobes which are presumably endopodites. For instance, in Philaenus these lobes are incised longitudinally into mesal and lateral portions, and in Agrion also they develop a pair of processes on their inner margins.

The inner pair of appendages of the ninth stermite in the female, though primarily free, are fused together in the last nymphal and in the adult stages in Philaenus.

The next important question of great morphological significance is the homology of the sub-genital plates. It has already been stated that Kershaw and Muir regarded them as coxites of the eighth sternum. According to Crampton they are 'hypovalvae' or extensions of the ninth sternum. Kershaw and Muir contend that the anterior ovipositor lobes and the sub-genital plates arise from similar situations behind the eighth sternum, and that the development of these structures in the two sexes is step by step identical till the last instar, when the sub-genital plates become fused to the ninth sternum. Their figures (5, 6, 8, 9, Pl. 13, 'Annals of Entomological Society of America', 1922) are cited as showing the gradual changes that occur. In fig. 5 they show 'g' (sub-genital plates) as belonging to the eighth sternum, and in figs. 8 and 9 they show them attached to the ninth. Fig. 6 shows a transition stage. The differences in these figures are so minute that slight shifting of the chitin pieces by pressure might easily produce them. Chitin preparations alone do not seem to me to be sufficient evidence for the conclusions arrived at.

Singh-Pruthi maintained that the sub-genital plates are definitely on the ninth sternum. In refuting Kerhsaw and Muir he says 'I have examined the' Homopterous female also, and fig. 6, Pl. 5 ('Quart. Journ. Micr. Sçi.', 1924, p. 74), illustrates a transverse section of its body in the region of the ninth segment. If this be compared with fig. 2, Pl. 5, which is of the male of the same age, it will be noticed (1) that the place of origin of the sub-genital plates and that of the anterior ovipositor lobes which should be the same if they are homologous, are not so, that of the latter being much anterior ; (2) that while the female has three pairs the male at this stage has only two ; (3) that the dorsal pair of ovipositor lobes have the same appearance and position as the male paramere lobes which are to develop later into both the aedeagus and 'parameres'. Obviously the first of Singh-Pruthi's objections to Kershaw and Muir's view is the most important of the three. To have compared transverse sections of the male and the female and then concluded that the anterior pair of the one is far anterior to that of the other is certainly not the ideal way of doing it. Vertical longitudinal sections should show this condition much better. I have examined longitudinal sections of the male and the female nymphs of the same age. Though there is a similarity in the appendages there is no doubt that in the male the first pair (sub-genital plates) is situated on the ninth and in the female (the anterior ovipositor lobes) on the eighth. Careful study of the longitudinal segmental muscles shows that in the male those of the eighth terminate anterior to the sub-genital plates, while in the female they terminate behind the anterior ovipositor lobes. This I believe is evidence enough to show that the conclusions of Kershaw and Muir are untenable. Moreover, there is apparently no insect in which the homologue of the anterior pair of ovipositor lobes persists in the male, except Machilis polypoda, in which there are two processes present on the eighth which might be taken to represent the anterior ovipositor lobes.

A few minor objections raised by Muir in reply to Singh-Pruthi may also be considered briefly. He maintains that Pruthi, having examined only the later instars, might have lost sight of the great changes that have already taken place. There is no evidence to show that such changes take place. Muir questions whether there is a ninth sternum in the early nymph. In the early stages the ninth sternum is mostly occupied by the genital groove and the appendages, and what is left is only a narrow strip towards the posterior edge of that segment. As the opening of the ejaculatory duct is on the ninth segment in insects in general, that region in Homoptera should be regarded as the ninth sternum.

There remains Crampton's view to be considered. The fact that, from the earliest stages, the sub-genital plates develop as sutured appendages, just like any other appendages, and not as mere extensions of the sternal plates, is against his view. Muir has raised the objection that the sub-genital plates, the specializations of the hypandrium according to Crampton, are least developed in the more specialized groups of Homoptera and most in primitive ones. It should be the opposite if sub-genital plates are prolongations of the hypandrium.

In conclusion the following homologies of the genitalia are arrived at :

Female.	Male.
1. Anterior ovipositor lobes, segment 8.	Absent, segment 8.
2. Inner ovipositor lobes, segment 9.	Aedeagus, segment 9.
3. Lateral ovipositor lobes, segment 9.	Sub-genital plates, segment 9.

Summary and Conclusions.

1. The ' Parameres ' of Homoptera are not structures of any morphological significance, as assumed by some authors, but are the outgrowths of the aedeagus.

2. The sub-genital plates of the male are coxites of the ninth and not of the eighth segment as Kershaw and Muir have stated.

3. The aedeagus arises as a pair of lobes in the young, and during the later stages these lobes fuse to form the copulatory organ.

4. A pair of secondary processes develops in the female dragon-fly from the inner faces of the anterior lobes.

5. The basi-valvulae in Homoptera presumably represents the coxites of the eighth segment.

6. The ovipositors of the Zygopteron and the Homopteron differ in the former being open on the ventral side while the latter forms a closed tube.

PART II. EFFERENT GENITAL DUCTS.

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INTRODUCTION.

THOUGH a good many investigators during their embryological and post-embryological studies have dealt with the efferent genital ducts, very few of them have undertaken a special study of the system as such. Consequently the literature dealing with it is not only scanty but imperfect. A comprehensive understanding of the homologies of the different structures constituting the efferent genital system and a correct interpretation of the probable lines of evolution, are thereby rendered difficult. Are the gonopores, the accessory glands, the common oviduct, homologous in the different groups? What structures of the male are homologous with those of the female? These questions are still not fully answered. This paper embodies the results obtained from an ontogenetic study of the genital ducts of the groups Homoptera and Zygoptera as shown in the life-history of Philaenus and Agrion, and the author proposes to study other groups subsequently.

Historical Survey.

Harold (1815) was the first to show that part of the female efferent system arises by invagination from the hypodermis (ectoderm). Later workers like Suckow and Bessels confirmed his findings. Weismann (1864) showed that, of the efferent genital ducts, only the vasa deferentia and the oviducts are mesodermal, all other structures being ectodermal. Palmen (1884), working on Ephemeroptera, arrived at the conclusion that this group possesses a pair of openings in the female. Nüssbaum (1884) found that in Blattidae and Pediculidae the uterus, the vagina, the receptaculum seminis, ductus ejaculatorius, accessory glands, and the penis develop from the ectoderm. In describing the development in the male he states that the accessory glands and the vesiculae seminales arise from two ectodermal thickenings which unite together posteriorly to form the ductus ejaculatorius. He found that in both the sexes the unpaired structures arise from paired rudiments ; therefore he homologized the parts of the male efferent system with those of the female. Hatchett Jackson (1889) found that in Lepidoptera the female paired ducts end on the seventh abdominal segment in the larva, and that the common oviduct is formed partly as a groove on the ninth sternum and partly from two invaginations behind sternites 8 and 9. He questioned Nüssbaum's statement that the common oviduct had a paired origin. In interpreting his results Jackson showed that these structures in Lepidoptera passed through a larval ' ephemerid ' stage and a pupal 'Orthopteron' stage before they attain the adult condition. Wheeler (1889 and 1893) found in the embryo of Blatta the posterior extremity of the vasa deferentia and the oviduct to be the coelomic diverticula of the tenth and the seventh abdominal segments respectively in the two sexes. These swollen ends of the gonoducts he termed ' ampullae '. He further showed that an ectodermal invagination from the abdominal segment 9 in the male and 8 in the female joins the gonoducts to effect an opening to the outside. Wheeler criticized Nüssbaum's idea of the ectodermal origin of the vesiculae seminales and of the paired origin of the uterus and of the ejaculatory duct. Escherich (1894), dealing with the two sets of accessory glands in the male beetle, described them as mesodenia and ectodenia, denoting thereby their meso- and ectodermal origins respectively. Verson and Bisson (1896) supported Wheeler in his main conclusions. Muir (1915 and 1918) supported Escherich as regards mesodermal and ectodermal origins of the two separate accessory glands in the male beetle. Christophers and Cragg (1922) found that in Cimex the vagina and the ductus ejaculatorius are of ectodermal origin and all other parts are mesodermal. Singh-Pruthi (1924) in the male Homopteron noticed a pair of ducts, which he took to be ectodermal, intervening between the vasa deferentia and the ejaculatory duct during development. In the male Coleopteron Singh-Pruthi ascribed the posterior swollen ends of the vasa deferentia to the ectoderm, and showed the accessory glands (mesodenia and ectodenia), arising as they do from the swollen ends of the vasa deferentia, to be of ectodermal origin. He homologized the posterior ectodermal region of the vasa deferentia with the paired ducts he noticed in Homoptera, and so came to the conclusion that, in insects in general, there is a pair of ectodermal ducts (paired ejaculatory ducts) arising between the unpaired ejaculatory duct and the vasa deferentia and that in lower insects, like Ephemeroptera and Dermaptera, the common ejaculatory duct is not developed at all. In the female Coleopteron, according to him, the common oviduct is formed of two invaginations from abdominal segments 8 and 9, and the latter, in addition, give rise to the sphermatheca. He homologized the invagination in the eighth abdominal segment with the paired ejaculatory ducts of the male and the invagination in the ninth with the unpaired ejaculatory duct.

MATERIAL AND METHOD. Same as in Part I.

Philaenus leucophthalmus, L.: Male.

There are two testes situated in a ventro-lateral position in the body-cavity in the fourth and the fifth abdominal segments. Each testis is made up of a number of lobules. Adjacent to the testis lies a peculiar tissue consisting of large cells with prominent nuclei and somewhat granular cytoplasm. The function of this tissue is apparently unknown. There is a pair of vasa deferentia which are narrow coiled tubes and, in the region of the eighth abdominal segment, these enlarge into vesiculae seminales. Thereafter they are continued as short ducts, and into them the accessory glands open. The two vasa deferentia, after receiving the accessory glands, open into an ejaculatory duct which runs into the aedeagus situated on the ninth segment. The posterior region of the vasa deferentia and the ejaculatory duct are covered by a layer of fibrous and muscular tissue. Fig. 18, Pl. 28, gives a diagrammatic sketch of the male organs.

Development.

In the earliest nymphal stage the testes are well differentiated, and the vasa deferentia are present as solid chords till they reach the ninth abdominal segment, where they are rather thick-walled and tubular. The tubular ends rest on the hypodermis of the rudimentary appendages of that segment. Figs. 4 and 5, Pl. 27, show transverse sections passing through the ninth abdominal segment, and the tubular extremity of the vasa deferentia are marked *am.vd.* On the sternal region of the ninth segment is a groove which denotes the region where the ejaculatory duct develops at a later stage.

In a later stage the invagination of the ejaculatory duct develops from the sternal groove referred to, and this invagination comes to lie between the posterior ends of the vasa deferentia. Fig. 19, Pl. 28, shows this. Mesoderm cells are to be seen gathering round these ducts at this stage. From the anterior ends of the tubular region of the vasa deferentia a protuberance develops forward, and this is the rudiment of the accessory gland. Soon the solid structure acquires a lumen, and figs. 20 and 21, Pl. 28, illustrate the origin of the accessory gland.

During the third instar the ends of the vasa deferentia and the ejaculatory duct come near each other and the rudiments of the accessory glands become more elongated and better developed and the vasa deferentia develop a lumen where they were previously solid. A little anterior to the budding of the accessory gland the vasa deferentia widen out a little. This indicates the future vesiculae seminales. The swollen extremities of the vasa deferentia have become more elongated and therefore more narrow. The development during the penultimate and last stages consists of the establishment of the communication between the vasa deferentia and the ejaculatory duct.

Agrion: Male.

There are two testes situated on the dorsal aspect of the alimentary canal and ventral to the heart and extending from the fourth to the eighth abdominal segment. The vasa deferentia originate from the mid region of the testes, and when they reach the eighth segment take an oblique course towards the ventral side. In the ninth segment they open into a vesicular sac. This sac opens to the outside between the bases of the coxites of that segment. Fig. 22, Pl. 29, illustrates the arrangement of the male organs in Agrion.

Development.

In the earliest nymphal stage the testes are well differentiated and are enclosed each in a thin membrane. The primary germ-cells are clearly marked, each having a prominent nucleus. The vasa deferentia are thin solid chords, except in the region of the ninth segment, where they fuse together.

At a later stage a groove appears at the bases of the gonopophyses of the ninth segment, and later on it takes the form of an invagination. This ectodermal invagination, as it extends gradually into the body-cavity, meets the fused vasa deferentia, and at a still later stage the invagination extends to the fused vasa deferentia and becomes connected with them. The distended portion of the fused gonoducts at the point where the invagination referred to joins them later develops into the vesicular sac already mentioned.

DISCUSSION.

The above-described mode of development of genital ducts is similar for the most part in the two groups Homoptera and Zygoptera. The mesodermal vasa deferentia end on the ninth segment where they are joined by an invagination of the ectoderm. Thus an opening is effected to the outside. The differences noted are: in the Homoptera the two vasa deferentia remain separate from one another, whereas in Zygoptera they fuse posteriorly in the region of the ninth abdominal segment; accessory glands arise from the anterior region of the swollen extremities of the vasa deferentia in Homoptera, whereas no accessory glands develop in Zygoptera.

Thus the study of the development of the efferent genital ducts in these two groups shows no sign of a pair of ectodermal ducts ending blindly in the body-cavity during the early nymphal stages, and afterwards fusing with the vasa deferentia anteriorly and with the median ejaculatory duct posteriorly. My results therefore differ from what Singh-Pruthi found in the Jassidae. Singh-Pruthi, laying stress on the presence of a pair of ectodermal ejaculatory ducts in insects in general, claims that Nüssbaum found a similar pair of ducts in Blattidae and Pediculidae, and criticizes Wheeler for stating that Nüssbaum has mistaken the distended ends of the vasa deferentia to be ectodermal in origin. Nüssbaum describes the median ejaculatory duct as having arisen from two ectodermal thickenings on the ninth abdominal sternite, and these ectodermal thickenings, as described by Nüssbaum, appear to be quite different from the slender ducts noted by Pruthi in Idiocerus, but are similar to the swollen extremities of the vasa deferentia or ‘ampullae’ as correctly pointed out by Wheeler. Moreover, Nüssbaum traced the vesiculae seminales to these 'ectodermal thickenings'. No subsequent worker has been able to support Nüssbaum as regards their ectodermal origin, Wheeler, Verson, and Bisson and others having noted their development from mesoderm. Since the ampullar region of the vasa deferentia is well marked off from the rest of the duct, and since the cells of the ampullae are considerably larger, and, working as he did with the aid of whole mounts without sections, Nüssbaum has, it appears, taken the ampullae to be the anterior extremities of a pair of hypodermal invaginations, and these according to him, while giving rise to the vesiculae seminales anteriorly, unite posteriorly to form the ductus ejaculatorius. And that is how he traced the ejaculatory duct to a pair of rudiments in the young.

Singh-Pruthi has compared the T-shaped ducts noted by Verson and Bisson in Lepidoptera to the paired ectodermal ejaculatory ducts, noted by him in Idiocerus. But the T-shaped ducts are not distinct ducts as such, as they develop as outgrowths from the ampullae and Verson and Bisson have stated in unmistakable terms their mesodermal nature.

Again, while the ectodermal paired ejaculatory ducts described by Singh-Pruthi in Jassidae end blindly in the body-cavity in the early stages, their homologues in Coleoptera are not a pair of free ducts but form the extremities of the vasa deferentia. The ducts noted in Idiocerus are long and thin, whereas their supposed homologues in Coleoptera are somewhat roundish bodies. Pruthi lays a good deal of emphasis on the fact that the cells constituting these swollen ends of the vasa deferentia in Coleoptera are of a different appearance from those of the rest of the duct, and he argues therefrom that they should have a different origin. The posterior extremities of the oviducts are also distended in a similar manner and show a cellular structure different from the rest of the duct, and yet Singh-Pruthi does not doubt their mesodermal origin. On the contrary, the swollen extremities of the vasa deferentia of Coleoptera show considerable resemblance to the 'ampullae' of Wheeler, and until Wheeler's observations, which were confirmed by Heymons, are disproved, it seems reasonable to ascribe a mesodermal origin to these structures.

Thus, the 'paired ectodermal ejaculatory' ducts of Idiocerus remain as distinct structures peculiar to that insect, no such pair having been noted in Cercopidae which, being a more primitive group, very probably would have retained them. On the other hand, these ducts, as drawn in Singh-Pruthi's figures, show a striking resemblance to the rudiments of accessory glands of Cercopidae and, as Singh-Pruthi did not examine early nymphal stages, he probably mistook the accessory glands for the 'paired ejaculatory' ducts. I could not verify it in Idiocerus, as I could not procure material, but hope to do so later.

Singh-Pruthi's fig. 1 *a* shows the vasa deferentia ending at the bases of the sub-genital plates on the ninth segment and the 'paired ejaculatory' ducts extending forward almost to the anterior limits of the eighth segment. How could the 'paired ejaculatory' ducts so situated fuse end to end with the vasa deferentia unless either set of them undergoes a twisting? Singh-Pruthi has not mentioned any such twisting. Nor is his fig. 4, which is meant to illustrate the fusion of the paired ejaculatory ducts with the vasa deferentia, clear on this point.

Further, Singh-Pruthi seems to think that the 'paired ejaculatory' ducts join the median ejaculatory duct before they join the vasa deferentia. The contrary is to be expected inasmuch as the paired ejaculatory ducts are supposed to be more primitive than the median ejaculatory duct.

In this connexion Singh-Pruthi's criticism of Wheeler's observations deserves some attention. He says: 'As regards Wheeler, it seems really curious that he, while believing that the primitive insects had a pair of terminal ectodermal ducts and that the single terminal ejaculatory duct met with in the present-day insects is a secondary integumental invagination and does not arise by the coalescence of the original pair of ducts, should fail to imagine the presence of a pair of ectodermal ducts intervening between the vasa deferentia and the terminal ejaculatory duct.' These paired ejaculatory ducts occur in

Ephemeroptera and some Dermaptera in which they enter the penis and join the vasa deferentia on the ninth abdominal segment. In the higher insects the paired penes have fused to form a median aedeagus, and the median ejaculatory duct was developed in place of the paired ducts, which were lost entirely. There seems to be no reason for believing that these paired ducts exist side by side with the median ejaculatory duct which has supplanted them, and to imagine that they have shifted anteriorly so as to lie between the vasa deferentia and the median ejaculatory duct—such a change should take place if Singh-Pruthi's view is to be accepted—is beyond reasonable conception. Therefore there seems to be little or no evidence for his view as to the existence of a pair of ectodermal ejaculatory ducts in Homoptera or in any other of the higher insects.

The accessory glands which develop in Philaenus from the anterior extremities of the ampullae of the vasa deferentia are mesodermal in origin, and this conclusion probably holds good for other groups as well. The ectodenia and mesodenia arising as they do from the ampullae, I take it that the distended ends of the vasa deferentia as such are mesodermal in origin and not ectodermal as Singh-Pruthi believes.

Philaenus leucophthalmus L.: Female.

The ovaries are situated in the fifth abdominal segment and, as in the case of the testes, are surrounded by a peculiar tissue. The two ducts open into a common oviduct, on the dorsal aspect of which opens the spermatheca. The spermatheca consists of a rather thick-walled sac and a very well-developed gland. The common oviduct, whose walls are also thick, is dilated into two hollow sacs, each of which shows a characteristic kidney-shaped appearance in transverse section. The epithelium of these sacs consists of columnar cells which appear to be glandular in function. The common oviduct, which is dilated into a vagina in the posterior region, opens to the outside between abdominal segments 8 and 9. On the ninth segment between the bases of the inner ovipositor lobes opens a median accessory gland. It consists of a long glandular body terminating in a thick-walled pouch which opens to the outside by means of a narrow, short duct. A little posterior to the vaginal opening are situated the openings of a pair of accessory glands, one on either side. Fig. 23, Pl. 29, shows the arrangement of the genital organs of the adult female.

Development.

In the first instar nymph the ovaries are easily recognizable. The oviducts are narrow inconspicuous solid chords which terminate in dorso-ventrally flattened ampullae on the seventh abdominal segment. Fig. 24, Pl. 29, is a transverse section of the first instar nymph showing the ampullae marked *am.o.d.* There is present a median ventral groove on the ninth abdominal segment at this stage.

During the second nymphal stage the ectodermal groove on the ninth segment develops in such a way as to result in an invagination extending anteriorly into the body-cavity. This is the rudiment of the unpaired or the median accessory gland, already mentioned. The gonapophyses on the ninth segment are also laid out as rudiments by this time. Towards the close of the second instar the beginning of a second invagination which is taking place between segments 7 and 8 shows itself. Fig. 25, Pl. 29, shows the ectodermal invagination between 7 and 8.

During the third instar the ectodermal invagination between segments 7 and 8 grows anteriorly so as to lie dorsal to the oviducal ampullae which have by this time markedly developed. Fig. 26, Pl. 29, illustrates the position of the organs at this stage. Towards the close of the third instar the median accessory gland becomes bent upon itself in the region of the eighth segment owing to the sternum of the ninth segment having telescoped slightly into the eighth. Further, a groove extending from the posterior margin of the seventh segment to the bases of the anterior ovipositor lobes of the eighth segment makes its appearance.

During the penultimate stage this longitudinal groove by fusion of its lips becomes converted into a tube, and thus the genital pore originally on the seventh sternum shifts backwards to the eighth. The ectodermal invagination from the seventh segment touches the oviducal ampullae and opens into them. There is thus established a single common duct which extends from the ampullae to the bases of the anterior ovipositor lobes on the eighth segment. The part of the invagination from the seventh which lies dorsal to the ampullae remains as an outgrowth from this median duct and becomes the spermatheca. Fig. 27, Pl. 29, shows the ectodermal groove, and fig. 28, Pl. 29, shows the closure of the groove to form a tube at the common oviduct. Figs. 29 and 30, Pl. 29, shows diagrammatically the development of the common oviduct and the consequent shifting of the vaginal aperture from the seventh to the eighth segment.

Agrion: Female.

The ovaries are situated in abdominal segments 3 and 4 on either side of, rather below, the heart. An oviduct emanates from each ovary and, after taking an oblique course downwards, enlarges into a small sac in the eighth segment. Behind this the two oviducts unite into a common duct which in the eighth segment receives the spermatheca on its dorsal side. The spermatheca consists of a gland and a wide sac or pouch. The gland, which lies closely applied to the sac, shows a triangular shape in cross-section. The spermathecal wall consists of two layers, an outer of round cells and an inner of columnar cells; in addition to these layers the cavity is lined with a structureless matrix. At the point where the spermatheca unites with the common oviduct there are two flaps, presumably valves. Beyond the spermatheca the oviduct proceeds posteriorly as a laterally compressed tube the walls of which are made up of conspicuous columnar cells with oval or round nuclei. The common oviduct is then continued into the vagina which opens between segments 8 and 9. On either side of the vaginal groove opens an accessory gland which lies in the ninth segment. Figs. 30 and 31, Pl. 29, illustrate these structures.

Before describing the development of the genital ducts I wish to pass a few remarks on the anatomy of Zygoptera. R. J. Tillyard, in his book on the biology of dragon-flies, gives a description with illustrations of the female genital organs of Petaluria gigantia (Anisoptera) which does not tally with my observations on Agrion (Zygoptera). He describes the two oviducts as independently opening into the spermatheca. I find the oviducts join before they open into the spermatheca. He shows two spermathecal glands, accessory sacs as he calls them, opening by a common duct into the spermathecal sac, but I find only a single gland of trifid appearance lying closely applied to the spermatheca proper. Tillyard makes no mention of the accessory glands opening into the vaginal groove.

Development.

In the early nymphs the ovaries are easily recognized. The oviducts occur as thin solid chords which, after taking an oblique course downwards, fuse together and terminate in the eighth segment. An ectodermal invagination arising between the bases of the anterior ovipositor lobes meets this common oviduct in the eighth segment and lies dorsal to it. During the later stages the oviducts acquire a lumen and the ectodermal invagination becomes more pronounced. The accessory glands develop as two ectodermal invaginations from the anterior region of the ninth segment. From the anterior region of the ectodermal invagination in the eighth segment an outgrowth develops which is the rudiment of the spermatheca. The rudiments of the accessory glands grow backward into the ninth segment.

During the later stages the upper wall of the common oviduct breaks down, and thus it comes to open into the ectodermal invagination which, as already stated, has extended forwards until its apex has passed above the oviduct. The spermathecal folds and gland develop from the outgrowth of the invagination which has already been referred to. Proliferation of cells on the inner face of each anterior ovipositor lobe takes place, and this ultimately results in the formation of two processes extending between the bases of the anterior and inner ovipositor lobes. The groove enclosed by these processes connects the vagina to the inner ovipositor lobe (vide figs. 16, 17, and 18, Pl. 28).

DISCUSSION.

The Female Gonopore.

In Rhynchota (Hemiptera), Odonata, Orthoptera, and Hymenoptera the female opening is situated between segments 8 and 9, and in Lepidoptera and Coleoptera between segments 9 and 10, though in Lepidoptera there is an additional opening of the copulatory pouch on the eighth segment. Berlese included the Heteropterous bugs and the Odonata in the second category, and Vander Weele also located the female opening in Odonata on the ninth segment. As already shown, the vaginal invagination in Agrion, which develops at a very early stage, is continuous anteriorly with the bases of the anterior ovipositor lobes and therefore strictly on the eighth segment. The invagination noted by Vander Weele on the ninth segment is perhaps that of the accessory glands and not of the vagina. Wheeler and Heymons assigned the female opening to the seventh abdominal segment in Orthoptera. Singh-Pruthi dismissed the views of these authors on the assumption that Wheeler was not sure of the segments. I have described above how, in Philaenus, an invagination occurs from behind the seventh segment and subsequently fuses with the oviducts. It is, I believe, reasonable to assume that, in Orthoptera, Wheeler noted a similar happening, and as he did not go in detail into the later development concluded that the invagination from the seventh was that of the vagina.

A comparison of Homoptera with Odonata on this issue is of some interest. While there is no trace of any invagination on the seventh segment in Odonata in the nymphal stages, there is one in Homoptera during the second instar. In other insects in which such an invagination has been noted it is always observed to occur in the embryonic stages (Berlese). Is the single oviduct which is continuous with the paired duct in the early nymphal stages formed from an invagination from the seventh segment in Odonata ? The structure of the paired ducts and of the single duct is so uniform that it seems obvious that they belong to the same tissue, in other words the single duct must be mesodermal. The significance of these differences in the two groups will be considered later.

The Common Oviduct and the Vagina.

It has been stated above that in the early decade of the last century Harold, Suckow, Witlaczil, and Balbiani established that the terminal portion of the female efferent system is of ectodermal origin, and that Nüssbaum, while agreeing with the previous workers as to the ectodermal origin of the paired oviduct, traced that structure in Blattidae and Pediculidae to a pair of rudiments in the early stage. Nüssbaum's views have not had a general acceptance amongst subsequent workers, and embryologists like Wheeler recorded their emphatic dissent. Nevertheless Singh-Pruthi working on Tenebrio molitor, though he did not himself find the paired origin of the common oviduct, maintained its primitive paired nature, believing the single condition in Coleoptera to be a secondary modification. It has been stated in connexion with the development of the median ejaculatory duct that Nüssbaum's findings on the paired origin of that organ have been universally discredited. The development of the common oviduct in Homoptera and Odonata as described above lends no support to Nüssbaum's view. Jackson working on Lepidoptera found that the ectodermal invaginations behind the seventh and ninth segments both have a paired nature. His interpretation is founded on the fact that these invaginations apparently became double immediately after they had commenced to develop. Later, Jackson definitely states that no part of the common oviduct proper shows a paired origin. Verson and Bisson describe the vagina as arising from two folds from the anterior ends of the imaginal discs of the eighth segment, the folds fusing together to form a single duct. They also thus differ from Nüssbaum. Singh-Pruthi's acceptance of Nüssbaum's view, although he himself found no evidence in support of it, is possibly due to his having homologized the common oviduct with the 'ectodermal paired ejaculatory ducts' and his consequent assumption of the double origin of the former.

Homology of the Different Structures.

The development of the ectodermal regions of the efferent system of the female shows that the unpaired oviduct and the accessory organs are not homologous in the different groups.

In Odonata I have already shown the presence of a posterior single oviduct of structure similar to the paired oviducts. In the developmental stages of the male it has been stated that the two vasa deferentia unite on the ventral side of the ninth segment by their extremities; I presume that the single terminating region of the female mesodermal ducts is the resultant of such a fusion of the two oviducts, not end to end but side by side. To this mesodermal duct the invagination from the eighth segment becomes fused. The unpaired oviduct is thus derived partly from the mesoderm and partly from the ectoderm.

On the other hand, the unpaired oviduct in Homoptera and probably in all other groups is purely ectodermal. But the different groups differ in the mode of development of the common duct. In Homoptera this oviduct is a derivative of the ectoderm of the seventh and of the eighth abdominal segments. In Lepidoptera it is derived from the eighth and ninth abdominal segments. There is no direct evidence to show that there is any participation of the seventh segmental ectoderm in the formation of the common duct in Lepidoptera, but Jackson's description of the formation of the anterior region of the common oviduct betrays some doubt as to whether an invagination has not taken place on the seventh segment. He says on p. 162: 'The development of the second portion is very rapid indeed. The hollow base upon which the first vesicle is raised extends forwards, but it does so apparently only for a certain distance. At present exact details as to the mode of growth at the apex of this portion are not clear to me. This much is certain, that while near the base of the vesicle there is a tube with walls formed of a single layer of cells, the tube as it nears the larval oviducts becomes solid, and is more or less confounded with the ectoderm. My own impression is that there is an ingrowth from the ectoderm of cells which subsequently arrange themselves in the form of a tube; in other words the invagination of cells is at first solid.' On the other hand, Singh-Pruthi has shown that the common oviduct is derived from two invaginations, one from the eighth and the other from the ninth abdominal segments in Coleoptera. As stated above, these data show clearly that the common oviduct in the groups dealt with is not homologous.

The spermatheca was regarded by earlier workers, such as Witlaczil, Balbiani, and others, to be an outgrowth of the vagina or, in other words, of the invagination from the eighth abdominal segment. This view is correct of Odonata, Hymenoptera, and Lepidoptera. Singh-Pruthi maintains that this structure always arises from the invagination behind the ninth segment. This is true of Coleoptera and some Orthoptera. The Homoptera, however, do not fit in either of these groups since the invagination which gives rise to the spermatheca is on the seventh segment. In fact invaginations arising from any one of the segments 7, 8, or 9 may give rise to the spermatheca which therefore is not homologous in the different groups. On the other hand, the median accessory gland of Homoptera, the subaceous gland of Lepidoptera, and the accessory gland in Isoptera are homologous with the spermatheca of Coleoptera and some Orthoptera (Locustidae (Acridiidae) and Blattidae). The bursa copulatrix on the ninth segment in some Diptera (Christophers) also appears to be of the same status as the above-mentioned organs.

The paired accessory glands of Odonata and Homoptera make their appearance as paired invaginations on either side of the vagina, on the anterior region of the ninth abdominal segment. The homology of these glands is not very clear; they are probably homologous with the odoriferous glands of Lepidoptera. The pygidial or anal glands of insects, where they occur, arise in a similar manner to these accessory glands but from the tenth segment. I should differ from Berlese in his view that the pygidial glands are comparable to the median ectodermal invaginations from the genital segments.

In text-books of entomology it is customary to homologize the ejaculatory duct of the male with the unpaired oviduct of the female. Singh-Pruthi takes objection to this and, according to him, the homologue of the ejaculatory duct is the ectodermal invagination from the ninth segment in Coleoptera and Orthoptera (i. e. the ultimate spermatheca). I fully agree with him in this, but I do not subscribe to his view that the uterus (invagination on the eighth) is homologous to the 'paired ectodermal ejaculatory' ducts, for I question their existence in the higher Insecta. The accessory glands of the male, derived as they are from the mesoderm, are not homologous with the accessory glands of the female, which fact has already been pointed out by Verson and Bisson for Lepidoptera.

Probable Lines of Evolution of the Female Efferent System.

Though sufficient ontogenetic evidence is not available I shall hazard a provisional scheme showing the lines of evolution of the female efferent genital ducts in the different groups of Insecta. It is unquestionably admitted that the Ephemeroptera with their double female openings on the seventh abdominal segment exhibit a very ancient condition. This condition is still shown by many higher insects during their larval and nymphal stages.

The existence of an ectodermal invagination behind the seventh abdominal segment in Homoptera, Orthoptera, and probably Lepidoptera during ontogeny shows that the acquisition of a single gonopore was the first step in the line of evolution.

The later ontogenetic history shows that there has been a tendency to shift the gonopore to the terminal abdominal segments. This must have been established first by the formation of a groove extending from the seventh to the eighth abdominal segment. Gradually the groove was changed into a tube. Homoptera and Lepidoptera (Verson and Bisson) show, during their nymphal and pupal stages, respectively, the formation of a groove from the seventh to the eighth and its subsequent closure to form a tube.

The tendency to shift the gonopore farther back was still in force, and the calling in of a hypodermal groove between segments 8 and 9 was the next step. This groove is still retained by the Homoptera in which, though the gonopore opens behind the eighth segment, a shallow groove runs up from the eighth segment to the bases of the inner ovipositor lobes on the ninth segment. In Orthoptera, by the overgrowth of the eighth sternum and the telescoping of the ninth into the eighth, the opening on the ninth has been placed in close association with that on the eighth. In Diptera and Hymenoptera, by the shrinkage of the sternal region between the invaginations on the eighth and the ninth, the opening on the ninth has been shifted forwards so as to lie in touch with that on the eighth.

Lepidoptera show the highest stage in evolution in having converted the groove from the eighth to the ninth into a tube and in having retained the opening on the eighth.

Coleoptera followed quite a different path, the invagination from the ninth becomes confluent with invagination from the eighth and the opening on the eighth has been obliterated.

There is no doubt that the Odonata followed a different line altogether. There is no evidence of their ancestors having had an external opening on the seventh segment, because during the nymphal stages the mesodermal ducts join together and terminate in the middle of the eighth segment. Very probably the original female opening was in the middle of the eighth segment, and it has been shifted backwards to the intersegmental region between the eighth and the ninth subsequently. The development of the two curious processes, mentioned already, between the bases of the anterior ovipositor lobes, and the virtual establishment of communication between the gonopore and the ovipositor lobes of the ninth segment by means of the groove between these processes is quite unique in Insecta. In all these respects Odonata stands as a class by itself.

Specialization in the development of the accessory structures has taken place in different ways in the different groups. Homoptera developed unpaired accessory structures from the seventh and the ninth segment, Orthoptera (e.g. Gryllidae and Tettigoniidae) from the eighth, other Orthoptera (Locustidae

(Acridiidae) and Blattidae), Isoptera, Lepidoptera, and Diptera from the eighth and the ninth, Odonata from the eighth, and Coleoptera from the ninth. Paired glands were also developed in almost every group. The occurrence of an ectodermal accessory structure in association with the inner ovipositor lobes seems to be a very general occurrence in the higher Insecta, and the significance of this structure has not received sufficient attention at the hands of entomologists. Whether this structure was developed originally to function as a gland and afterwards became changed to act as a spermatheca in some groups or vice versa, only further study will enable us to understand. But one thing is certain, that it has been developed almost at the same time as the conversion of the appendages of the ninth segment to the ovipositor lobes. In Homoptera, Lepidoptera, Hymenoptera, and Isoptera it serves as a gland, in Orthoptera and Coleoptera as a spermatheca, in some Diptera it functions as a copulatory pouch, whereas in others (Chironomus) as a gland (Christophers).

SUMMARY AND CONCLUSIONS.

1. In the male Philaenus and Agrion the vasa deferentia terminate on the ninth segment in the early stages. An ectodermal invagination from that segment joins them subsequently and thus the male gonopore is established.

2. The accessory glands develop in Philaenus male from the anterior end of the swollen extremities of the vasa deferentia and the vesiculae seminales from a still more forward region.

3. The accessory glands of the male are mesodermal in origin and not ectodermal as some authors state.

4. There is no evidence as to the existence of a 'pair of ectodermal ejaculatory' ducts either in Philaenus or in Agrion, and reasons are adduced to show that they do not exist at all in the higher Insecta.

5. In the female nymph of Philaenus the oviducts terminate on the seventh segment. They are subsequently joined by an ectodermal invagination from the seventh segment. The common oviduct is formed in two parts: the anterior part is

derived from the posterior region of the invagination on the seventh and the posterior region is formed as a groove from the ectodermis of the eighth segment and subsequently this groove is converted into a tube. When the second part is completed it is in connexion with the invagination from the seventh and opens to the outside on the eighth segment. The ectodermal invagination from the seventh also gives rise to the spermatheca. A median accessory gland develops as an invagination from the ninth segment between the bases of the inner ovipositor lobes. A pair of accessory glands develop as paired invaginations from the anterior region of the ninth segment.

6. In the female nymph of Agrion the oviducts fuse to form a single duct and terminate in the middle of the eighth segment. Posteriorly an ectodermal invagination from the eighth segment meets this duct and lies in a position dorsal to it. Later on the ectodermal invagination develops a spermatheca dorsally and the mesodermal and the ectodermal ducts unite into one. The accessory glands develop as paired ectodermal invaginations from the anterior region of the ninth segment.

7. The female gonopore is not homologous in the different groups of insects. The vaginal opening in Orthoptera, Hymenoptera, Homoptera, Diptera, and Lepidoptera is homologous. The vaginal opening in Coleoptera is homologous with the oviducal opening of Lepidoptera, with the opening of the accessory gland of Homoptera, Hymenoptera, Diptera, Isoptera, and the opening of the spermatheca in some Orthoptera.

8. The common oviduct, being formed differently in the different groups is not homologous. The accessory organs, e. g. spermatheca, are not homologous in the different groups.

9. There is no evidence to show that the common oviduct is of paired origin.

10. The occurrence of a median accessory structure on the ninth segment which develops in the young as an invagination between the bases of the inner ovipositor lobes is very general in the higher Insecta. In some it functions as a gland, in others as a storehouse for spermatozoa.

11. The homology of the paired accessory glands is indicated.

12. The male genital ducts are not strictly homologous with those of the female. The homologue of the ejaculatory duct is the invagination from the ninth segment in the female.

13. The Odonata stand isolated in having a mesodermal region for the common oviduct and in the peculiar development of the two processes between the anterior ovipositor lobes.

14. The probable lines of evolution of the female efferent system in Insecta are indicated. The study of the development of the female efferent system indicates that the groups Orthoptera, Homoptera, Lepidoptera, and Diptera are very closely allied. Coleoptera seem to have had quite a different line of evolution from the above groups in this respect.

15. The adult Odonatan anatomy of the genital organs in the female as observed by me is in some respects different from that described by Tillyard.

In conclusion I wish to express my deep sense of gratitude towards Professor Balfour-Browne and Dr. J. W. Munroe, both of whom have always been ready to help me. My colleague Mr. R. I. Nel, who is working on similar lines in this department, has rendered me valuable help, not only in matters connected with the subject proper but also in translating difficult German references. I am also indebted to Mr. Peter Gray who helped me a good deal in translating references in Italian.

BIBLIOGRAPHY.

1. Balbiani, E. G.—" Contribution à l'étude de la formation des organes sexuels chez les insectes ", ' Recueil Zool. Suisse ', ii, 1885, pp. 527–88.
2. —— ' Phylloxéra du chêne et le Phylloxéra de la vigne.' Paris, 1884.
3. Balbiani, M.—" Mémoire sur la génération des Aphides ", ' Ann. des Sc. Nat.' (5), xiv, 1870 ; xv, 1872.
4. Berlese, A.—" Ricerche sugli organi genitali degli Ortotteri ", ' Atti della R. Accad. dei Lincei ', ser. 3 a, xi, 1881–2.
5. —— ' Gli Insetti ', i, 1909.
6. Bessels, E.—" Studien über die Entwicklung der Sexualdrüsen bei den Lepidopteren ", ' Zeit. f. wiss. Zool.', xvii, 1867.
7. Brüel, L.—" Anatomie und Entwicklungsgeschichte der Geschlechtsausfuhrwege sammt Annexen von Calliphora erythrocephala '. Zool. Jahrb. Anat. X.

8. Cholodkovsky, N.—" Ueber den Geschlechtsapparat von Nematois metallicus ", ' Zeit. f. wiss. Zool.', xlii, 1885, pp. 559–69.

9. Christophers, S. R., and Cragg.—" The development of the so-called penis, &c., of the bed-bug, Cimex lectularius, L.", ' Ind. Journ. Med. Res.', January 1922.

10. Christophers, S. R.—" The male genitalia of Anopheles ", ibid., iii, 1915, pp. 371–99.

11. —— " The development of the male Hypopygium of Diptera Anopheles ", ibid., October 1922.

12. —— " The development of the Terminal abdominal segments of the Mosquito ", ibid., 1922.

13. Christophers, R. R.—" The development of the terminal abdominal structures of the Sand-fly Phlebotomus ", ibid., 1926.

14. Crampton, G. C.—" Genitalia of lower insects ", ' Bull. Brooklyn Ent. Soc.', xiii, 1918, pp. 49–68.

15. —— " The Genitalia and Terminal Abdominal Structures of Male Neuroptera and Mecoptera, with Notes on Psocidae, Diptera, and Trichoptera ", ' Psyche ', xxv, 1918, pp. 47–59.

16. —— ' A comparison of the Genitalia of Male Hymenoptera, Mecoptera, Neuroptera, Diptera, Trichoptera, Lepidoptera, Homoptera, and Strepsiptera, with those of lower Insects ', ibid., xxvii, 1920, pp. 34–46.

17. —— " Remarks on the Basic Plan of the Terminal Abdominal structures of the Males of Winged Insects ", ' Canadian Ent.', lii, 1920, pp. 178–83.

18. —— " Correction of a statement concerning the Terminal Abdominal Structures of Male Insects ", ibid., liii, no. 3, 1921, p. 72.

19. —— " Preliminary notes on the Interpretation of Insectan and Myriopoden Structures through a Comparison with the Structures of Crustacea ", ' Trans. Ent. Soc. London ', 1921, pp. 340–6.

20. —— " Genitalia of Sawflies ", ' Proc. Ent. Soc. Washington ', xxi and xxiii, 1919 and 1921.

21. —— " The Genitalia of the Males of certain Hemiptera (Heteroptera) and Homoptera ", ' Bull. Brooklyn Ent. Soc.', 1922.

22. —— " The Genitalia of the Males of Homoptera ", ibid., 1922.

23. Dewitz, H.—" Ueber Bau und Entwicklung des Stachels und der Legescheide einiger Hymenopteren und der grünen Heuschrecke ", ' Zeit. f. wiss. Zool.', xxv, 1874–5, pp. 175–200.

24. Dufour, Léon.—' Recherches sur les Hémiptères ', 1834.

25. —— " Recherches anatomiques et physiologiques sur les Orthoptères, les Hyménoptères et Neuroptères ", ' Mém. de l'acad. des sc. sav. étrangers ', 2e sér., vii, 1841, pp. 404.

26. Eaton, A. E.—" Remarks upon the Homologies of the Ovipositor ", ' Trans. Ent. Soc. London ', 1868, pp. 141–4.

27. Edwards, F. M.—" The Nomenclature of the parts of the Male Hypopygium of Diptera, especially Mosquitoes ", 'Ann. Trop. Med. Liverpool', xiv, 1920, pp. 28–40.

28. Escherich, K.—" Beiträge zur Kenntnis der Thysanuren ", 1. Reihe, 'Zool. Anz.', xxvi, 1903, pp. 345–66.

29. —— " Das System der Lepismatiden ", 'Zoologica', xviii, 1905, pp. 1–149.

30. —— " Die biologische Bedeutung der Genitalanhänge der Insekten ", 'Verh. k. k. Zool.-Bot. Ges. Wien', xlii, 1872, pp. 225–40.

31. —— " Anatomische Studien über das männliche Genitalsystem der Coleopteren ", 'Zeit. f. wiss. Zool.', lvii, 1894, pp. 620–41.

32. Hasse, E.—" Die Abdominalanhänge der Insekten mit Berücksichtigung der Myriopoden ", 'Morph. Jahrb.', xv, 1889, pp. 331–435.

33. —— " Die Zusammensetzung des Körpers der Schaben ", 'Blattidae', Sitz. d. Gesell. Natur. Freunde zu Berlin', Juni 1889.

34. Harold.—'Entw.-Gesch. der Schmetterlinge.' Cassel und Marburg, 1815.

35. Heymons, R.—" Ueber den Genitalapparat und die Entwicklung von Hemimerus talpoides, W.", 'Zool. Jahrb.', Suppl. xv, no. 2, pp. 141–84.

36. —— " Die Entstehung der Geschlechtsdrüsen von Phyllodromia (Blatta) germanica ". Inaugural dissertation. Berlin, 1891. ('Zeit. f. wiss. Zool.', liii, Suppl., 1892, pp. 434–537.)

37. —— " Zur Morphologie der Abdominalanhänge bei den Insekten ", 'Morph. Jahrb.', xxiv, 1896, pp. 178–204.

38. —— " Der morphologische Bau des Insektenabdomens ", 'Zool. Centrbl.', vi, 1899, pp. 537–56.

39. —— " Beiträge zur Morphologie und Entwicklungsgeschichte der Rhynchoten ", 'Acta Acad. Leop.-Carol.', lxxiv, 1899, pp. 349–56.

40. Holmgren, N.—" Beiträge zur Kenntnis der weiblichen Geschlechtsorgane der Cicadarien ", 'Zool. Jahrb.', Abth., Syst. xii, 1899.

41. Imms, A. D.—'Text-book of Entomology,' 1924.

42. Jackson, H. W.—" Studies in the Morphology of the Lepidoptera ", 'Zool. Anz.', 1889; and 'Trans. Linn. Soc. London', 2nd ser. v, Zoology, 1888, pp. 143–67.

43. Kershaw, J. C., and Muir, F.—" The Genitalia of the Auchenorhynchous Homoptera ", 'Ann. Ent. Soc. America ", xv, no. 3, 1922, pp. 201–12.

44. Knower, H. McE.—" A comparative study of the development of the Generative Tract in Termites ", 'Johns Hopkins Hospital Bulletin', xii, 1901, p. 135.

45. Kraepelin, C.—" Untersuchungen über den Bau, Mechanismus und

Entwicklungsgeschichte des Stachels der bienenartigen Thiere ", ' Zeit. f. wiss. Zool.', xxiii, 1873.

46. Lacaze-Duthiers, H.—" Recherches sur l'armure génitale des Insectes ", ' Acad. d. Sc. Nat.', 3rd ser., Zool., t. xii–xix, 1849–53.

47. Lowne, B. T.—" On the structure and development of the ovaries and their Appendages in the Blow-fly (Calliphora erythrocephala) ", ' Journ. Linn. Soc. Zool.', xx, 1890, pp. 418–40.

47*a*. —— ' The Blow-fly.' 2 vols. London, 1890–2.

48. Muir, F.—" Anterior processes of the Male Genitalia of Homoptera ", ' Proc. Haw. Ent. Soc.', 1925 and 1926.

49. Nüssbaum, J.—" Zur Entwicklungsgeschichte der Ausführungsgänge der Sexualdrüsen bei den Insekten ", ' Zool. Anz.', v, 1882, and Lemberg, 1884.

50. Palmen, J. A.—' Über paarige Ausführungsgänge der Geschlechtsorgane der Insekten.' Helsingfors, 1884.

51. Saling, Th.—" Zur Kenntnis der Entwicklung der Keimdrüsen von Tenebrio molitor L.", ' Zeit. f. wiss. Zool.', lxxxvi, 1907, pp. 238–303.

52. Sharp, D., and Muir, F.—" The comparative anatomy of the Male genital tube in Coleoptera ", ' Trans. Ent. Soc. London ', 1912.

53. Singh-Pruthi, H.—" Development of the Male Genitalia in Homoptera with Preliminary Remarks on the Nature of these organs in other Insects ", ' Quart. Journ. Micr. Sci.', vol. 69, 1924.

54. —— " Morphology of the Male Genitalia in Rhynchota ", ' Trans. Entom. Soc. London ', 1924.

55. —— " On the Post Embryonic Development and Homologies of the Male Genital Organs of Tenebrio molitor L. (Coleoptera) ", ' Proc. Zool. Soc. London ', 1924.

56. —— " On the development of the Ovipositor and the efferent Genital Ducts of Tenebrio molitor L. (Coleoptera) with remarks on the comparison of the latter organs in the two sexes ", ibid., 1924.

57. Suckow.—' Anat.-phys. Untersuchungen der Insecten und Krustenthiere ', Th. i, Heidelberg, 1818. Also see " Geschlechtsorgane der Insecten ", Heusinger's ' Zeit. f. d. Organ. Physik ', 1828, pp. 231–64.

58. Tillyard, R. J.—' Biology of Dragon-flies.' Cambridge, 1917.

59. Verhoeff, C.—" Zur Vergleichenden Morphologie der Coxalorgane und Genitalanhänge der Tracheaten ", ' Zool. Anz.', xxvi, 1903, pp. 60–77.

60. —— " Bemerkungen über abdominale Körperanhänge bei Insekten und Myriapoden ", ibid., xx, 1897, pp. 293–300.

61. —— " Finden sich für die Laminae basales der männlichen Coleopteren Homologa bei Hymenopteren ? ", ibid., xvi, 1893.

62. —— " Über Felsenspringer, Machiloidea. 3. Aufsatz: Die Entwicklungsstufen ", ibid., xxxvi, 1910, pp. 385–97.

63. —— " Vergleichende Untersuchungen über die Abdominalsegmente und die Copulationsorgane der männlichen Coleopteren ", ' Deutsch. Ent. Zeit.', 1893, pp. 113–70.

64. Verson, E., and Bisson, E.—" Die postembryonale Entwicklung der Ausführungsgänge und der Nebendrüsen beim männlichen u. weiblichen Geschlechtsapparat von Bombyx mori ", ' Zeit. f. wiss. Zool.', lxi, 1896, pp. 318–37, and 660–94.

65. Walker, E. M.—" The Terminal Abdominal Structures of the Orthopteroid Insects—Female ", ' Ann. Ent. Soc. Amer.', 1919.

66. —— " The Terminal Abdominal Structures of the Orthopteroid Insects—Male ", ibid., 1922.

67. Weele, H. W. van der.—" Morphologie und Entwicklung der Gonapophysen der Odonaten ", ' Tijd. Ent.', xlix, 1906, pp. 99–198.

68. Wheeler, W. M.—" The Embryology of Blatta germanica, &c.", ' Journ. Morph.', iii, 1889.

69. —— " A contribution to Insect Embryology ", ibid., viii, 1893.

70. Weismann, A.—" Die nachembryonale Entwicklung der Musciden nach Beobachtungen an Musca vomitoria und Sarcophage carnaria ", ' Zeit. f. wiss. Zool.', xiv, 1864.

71. —— " Die Metamorphose der Corethra plumicornis ", ibid., xvi, 1866.

72. Witlaczil, E.—" Entwicklungsgeschichte der Aphiden ", ' Zeit. f. wiss. Zool.', xl, 1884, pp. 559–687.

73. Zander, E.—" Beiträge zur Morphologie der männlichen Geschlechtsanhänge der Hymenopteren ", ibid., lxvii, no. 3, 1900.

74. —— " Beiträge zur Morphologie der männlichen Geschlechtsanhänge der Lepidopteren ", ibid., lxxiv, 1904–5, pp. 557–615.

EXPLANATION OF PLATES 27, 28, 29.

LETTERING.

aed., aedeagus ; *aed-h.*, aedeagus hook ; *aed-r.*, aedeagus ridge ; *am.o.d.*, ampulla of the oviduct ; *am.v.d.*, ampulla of the vas deferens ; *a.o.l.*, anterior ovipositor lobe ; *a.s.*, anal style ; *b.v.*, basi valvula ; *c.*, coxite ; *cl.*, cleft ; *c.o.d.*, common oviduct ; *ct.*, chitin ; *ejd.*, ejaculatory duct ; *f.a.o.l.*, fold of the anterior ovipositor lobe ; *f.o.*, female opening ; *gr.*, groove between the processes of the anterior ovipositor lobe ; *h.*, ectodermis ; *h.g.*, ectodermal groove ; *h.gr.c.o.d.*, ectodermal groove forming the common oviduct ; *h. in* 7, ectodermal invagination from the seventh segment ; *i.o.l.*, inner ovipositor lobe ; *int.*, intestine ; *l.o.l.*, lateral ovipositor lobe ; *m.*, muscle ; *m.ac.g.*, median accessory gland of the female ; *m.o.*, male opening ; *o.*, ovary ; *o.d.*, oviduct ; *o.m.ac.g.*, opening of the median accessory gland of the female ; *o. in* 7, opening of the invagination from the seventh ; *p.ac.g.*, paired accessory gland ; *p.a.o.l.*, processes of the anterior ovipositor lobe ; *ph.ac.g.*, pouch of the accessory gland ; *p.l.*, penis lobe ; *pr.*, paramere ; *pr.b.*, primitive bud ; *r.aed.*, rudiment of the aedeagus ; *r.a.g.*, rudiment of the accessory gland of the male ; *r.a.ol.*, rudiment of the anterior ovipositor lobe ; *rd.o.l.*, ridge of the anterior ovipositor lobe; *r.i.o.l.*, rudiment of the inner ovipositor lobe; *rd.i.o.l.*, ridge of the inner ovipositor lobe; *r.l.o.l.*, rudiment of the lateral ovipositor lobe; *r.p.a.o.l.*, rudiment of the processes of the anterior ovipositor lobe; *r.pr.*, rudiment of the parameres; *s.g.*, surgonopod; *s.g.p.*, subgenital plate; *s.p.*, spermatheca; *sp.gl.*, spermathecal gland; *sp.v.*, spermathecal valve; *st.*, stylus; *st.gr.*, sternal groove; *t.*, testis; *t.p.*, tergal process; *tr.*, trachea; *v.*, vagina; *v.d.*, vas deferens; *vl.*, vesicle; *v.s.*, vesicula seminalis.

PLATE 27.

Figs. 1–9.—Philaenus.

Fig. 1.—Ventral view of the male genitalia. ×40.

Fig. 2.—Ventral view of the female genitalia. ×40.

Fig. 3.—Transverse section through the posterior region of the body showing the attachment of the ovipositor lobes. ×90.

Fig. 4.—Transverse section of the early first instar male through the ninth segment showing the primitive pair of buds. ×300.

Fig. 5. Transverse section through the late first instar male showing the rudiments of the sub-genital plates and the penis lobes. 300.

Fig. 6.—Transverse section through the second instar male showing the formation of the aedeagus and the parameres. ×150.

Fig. 7.—Transverse section through the male nymph showing the rudiments of the aedeagus and the parameres being united at their bases. The gonapophyses are enclosed as it were by the tergal processes. ×130.

Fig. 8.—Transverse section through male nymph showing a more advanced stage than is shown in fig. 7. ×90.

Fig. 9.—Transverse section through the ninth segment of the first instar female showing the genital groove and the rudiments of the appendages. ×300.

PLATE 28.

Figs. 10, 11; 18–21.—Philaenus. Figs. 12–17.—Agrion.

Fig. 10.—Transverse section of the female second instar showing the formation of the rudiments of the lateral and inner pair of ovipositor lobes from the original single pair of bends on the ninth segment. ×250.

Fig. 11.—Transverse section of the female nymph showing the formation of the rudiments of the basic valvulae and the anterior ovipositor lobes from the single pair of buds on the eighth. ×200.

Fig. 12.—Ventral view of the last three abdominal segments of the adult male. ×25.

Fig. 13.—Lateral view of the last three segments of the adult female showing the genitalia. ×15.

Fig. 14.—Transverse section through the ovipositor showing the attachment of the lobes. ×250.

Figs. 15, 16, 17.—Three stages in the development of the processes of the anterior ovipositor lobes. ×100.

Fig. 18.—A diagrammatic sketch of the male generative organs.

Fig. 19.—Transverse section through the male nymph showing the position in the body-cavity of the ejaculatory duct and the ampullae of the vasa deferentia. × 150.

Fig. 20.—A diagrammatic sketch of the vas deferens ending in the ampulla.

Fig. 21.—A diagrammatic sketch showing the development of the accessory gland from the anterior region of the ampulla of the vas deferens.

PLATE 29.

Figs. 22; 31, 32.—Agrion. Figs. 23–30.—Philaenus.

Fig. 22.—A diagrammatic figure to illustrate the male reproductive system for Agrion.

Fig. 23.—A diagrammatic sketch to illustrate the female reproductive system.

Fig. 24.—Transverse section through the posterior region of the seventh segment of the first instar female showing the ampullae of the oviducts. × 200.

Fig. 25.—A sagittal section of the female nymph showing the development of the invagination from the seventh segment. × 70.

Fig. 26.—A transverse section through the seventh segment of the female third instar showing the position of the efferent ducts at that stage. × 70.

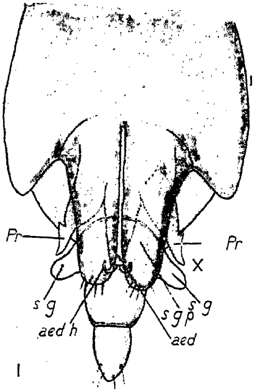
Fig. 27.—A transverse section through the eighth segment of the female nymph showing the groove which develops later on into the common oviduct. × 90.

Fig. 28.—A more posterior section to fig. 27 in which the groove has already been converted into a duct. × 90.

Figs. 29 and 30.—Diagrammatic illustrations to show the development of the common viaduct and the consequent shifting of the female opening from the seventh to the eighth.

Fig. 31.—A diagrammatic figure of the female reproductive organs of Agrion.

Fig. 32.—A transverse section through the eighth segment of female Agrion showing the vagina, spermatheca, and the spermathecal gland. × 80.



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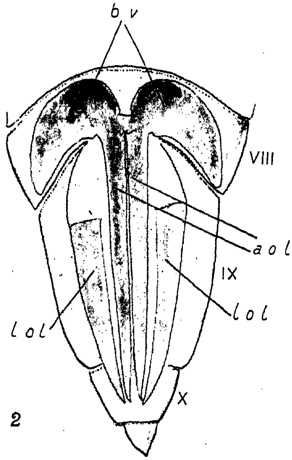

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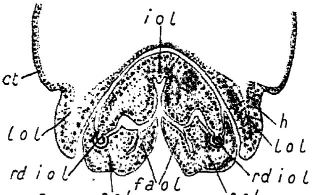

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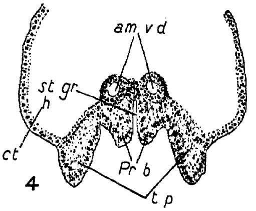

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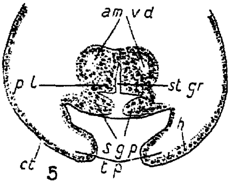

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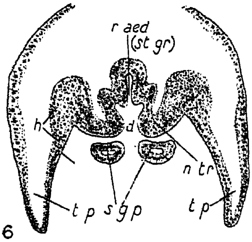

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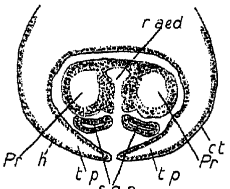

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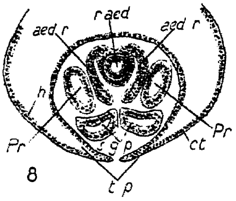

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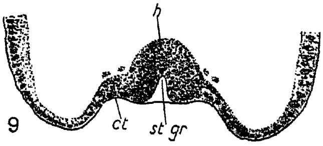

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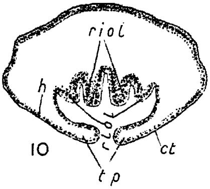

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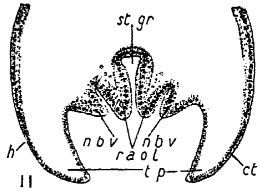

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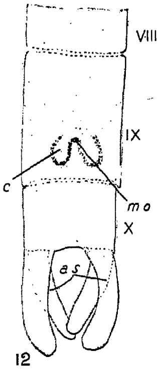

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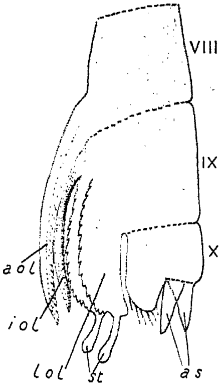

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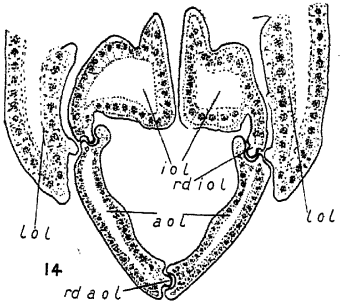

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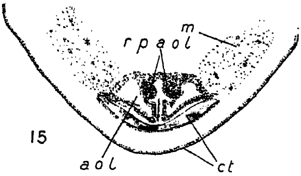

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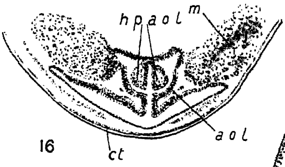

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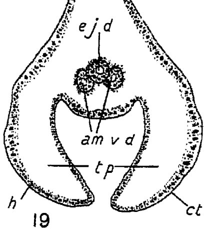

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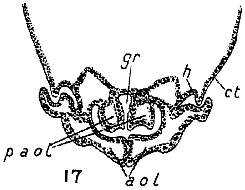

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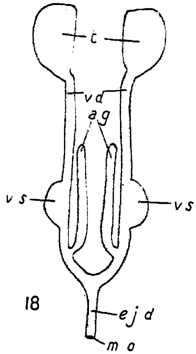

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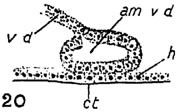

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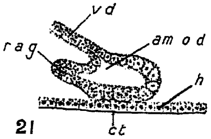

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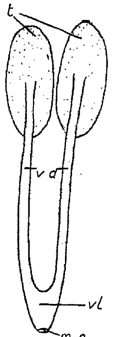

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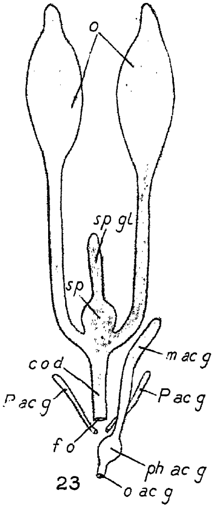

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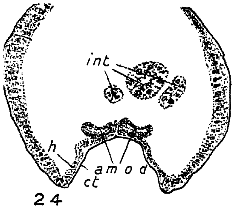

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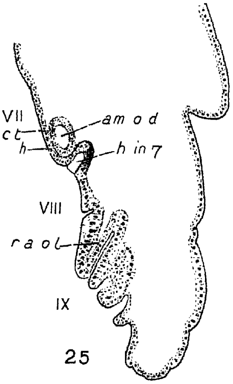

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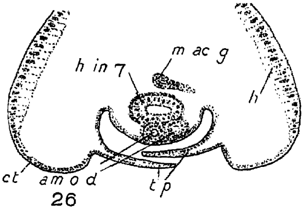

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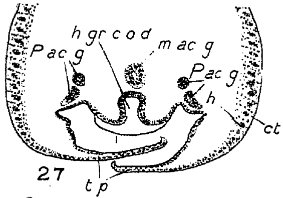

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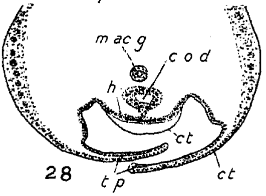

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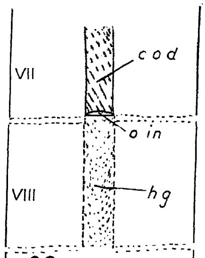

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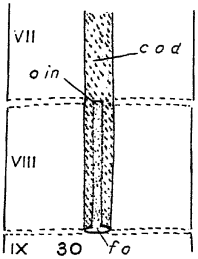

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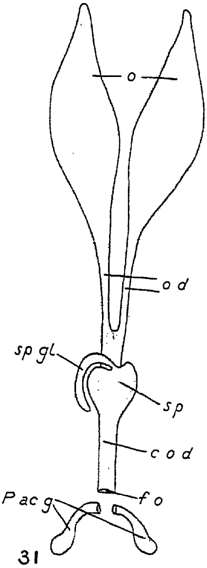

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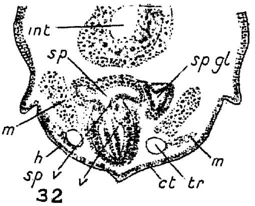

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C. J. George, del.

The Internal Anatomy of Lernaeopoda scyllicola Leigh-Sharpe.

Part I. The Female.

By

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From the Department of Zoology, Imperial College of Science and Technology, London.

With 12 Text-figures.

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INTRODUCTION.

Lernaeopoda scyllicola, a copepod parasitic upon Scylliorhynus (Scyllium) canicula, was first described by Leigh-Sharpe in 1916. Excellent as were his figures of the appendages and the external anatomy, those of the internal anatomy showed that much still remained to be learned. Fig. 6 of his paper shows no median spermatheca (though this, indeed, was previously stated by Wilson to be present in all the members of the family), while the 'cement gland' is shown as being the medial instead of the lateral wall of the oviduct. I draw these examples from my friend and teacher's paper, not from any desire to cast doubts upon his observational accuracy, but rather to show the unreliability of conclusions as to internal anatomy drawn from specimens mounted whole. What he figured as the ducts of the reproductive system is in reality the cement lying in these ducts. The spermatheca and these ducts are not normally visibly differentiated from the surrounding tissues, and in his specimens—through which, under his guidance, I first became acquainted with Lernaeopoda—are quite invisible. It was this apparent lack of internal organs which first led me to section Lernaeopoda, and it is upon reconstructions from serial sections that the major portion of this paper is based.

TECHNIQUE.

This work has been carried out upon twenty-five specimens obtained from the Plymouth Marine Biological Association. These specimens were fixed for fifteen minutes in a mixture of equal parts of glacial acetic acid, absolute alcohol, and chloroform, the whole saturated with corrosive sublimate. This is the only fixative which I have had tried for these copepods, and the results do not make me wish to experiment further.

The specimens were cut from blocks of paraffin melting at 58° C. into transverse, sagittal, and frontal sections, these latter not repaying the trouble required to obtain them. A thickness of 10μ was found to be the most useful.

With regard to staining I have carried out a very considerable range of experiments. Any form of haematoxylin and eosin (recommended by Wilson) has given me very poor results. Ehrlich's acid haematoxylin and acid fuchsin (recommended by Fasten) gave slightly better results. The best stain I have ever used is Mallory's connective-tissue stain with a slight modification as to the timing.[1] This gives nuclei red, muscle scarlet, ground-work of glands violet, epidermis blue, dermis red, matrix of ovary blue and egg-yolk red. The most important colour differentiation, however, is between the hardened and unchanged cements (vide subter), the former being blue and the latter red.

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[1] 1 per cent. acid fuchsin 5 minutes ; 1 per cent. phospho-molybdic acid 10 minutes ; Mallory's connective-tissue stain 20 to 30 minutes.

Where it is desired to show histological detail in the various organs rather than to differentiate between the different systems of organs I stain sections overnight in Grenacher's alcoholic borax carmine and pass directly from this into Ramon y Cajal's picro-indigo carmine for six to ten minutes. Nuclei are scarlet and everything else (including the egg-yolk) is pale green. This stain has the additional advantage of being less tiring to the eye than is Mallory's.

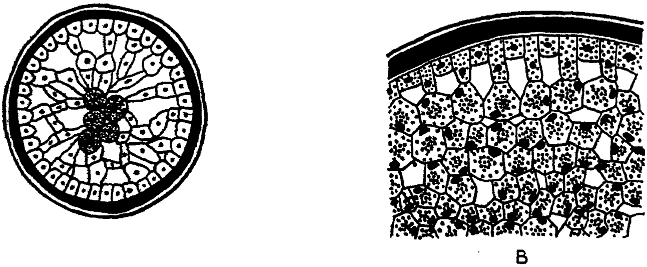
For specimens mounted whole it is better not to stain at all. Very little beyond the muscular system, which is almost perfectly displayed, can be made out in such specimens, and the most noticeable effect of staining is to diminish the transparency.

I invariably clear sections in xylol and whole specimens in oil of cedar-wood, finally mounting in balsam in xylol.

General Body Form.

As seen with the naked eye (Text-fig. 8) the parasite is divided into a posterior and an anterior region, which have been frequently referred to as the cephalothorax and the abdomen. These terms as applied to this animal obviously have very little relation to the same terms as applied to the higher Crustacea. Dr. Wilson (1915) is of the opinion that the posterior portion of the animal includes the 'posterior portion of the thorax, the genital segment, and the abdomen'. For this region he proposes the term 'trunk' which has accordingly been adopted throughout the remainder of this paper. The cephalothorax occupies about three-tenths of the entire length of the animal and bears two pairs of antennae, a pair of mandibles, two pairs of maxillae, and one pair of maxillipedes. For a consideration of the form and homologies of these the reader is referred to the original descriptions (Leigh-Sharpe, 1916). The trunk, averaging about half a centimetre long, bears at its posterior extremity a pair of 'abdominal appendages'. These are two sacs, which vary in size and shape according to the sexual condition of the parasite (vide subter), whose origin and homology appears to be very doubtful. Each is filled with a loose connective tissue in which lies a gland (Text-fig. 1 A). This gland is swollen[1] and apparently functional when the animal is about to produce egg-strings, and flaccid and exhausted when these strings have been produced. One is thus tempted to believe that the glands assist in this production; but this cannot be, as there is no channel by which the products of secretion could possibly reach the inside of any of the reproductive ducts since the gland opens directly into the lacunae which lie between the loose connective-tissue cells. We will return to this subject shortly.

TEXT-FIG. 1.



A. Diagram of abdominal appendage as seen in transverse section.
B. Diagram of portion of the food-reserve process as seen in transverse section.

The posterior end of the trunk (Text-fig. 11) is divided into four lobes by three dorso-ventral slits. The middle slit is the anus (*a*); from the two outer slits project the egg-strings (*e*). The vulvae open upon the ventral surfaces of the two inner lobes which are therefore the ' genital processes ' or, more correctly, the ' genital process ' split by the anus. The two outer lobes are occupied by closely-packed cells hexagonal in section (Text-fig. 1 B) filled with a granular substance; in my opinion they contain food reserves, and throughout the remainder of this paper will be referred to as the ' food-reserve processes '. The lacunae in these are in open communication with the lacunae of the abdominal appendages. Now it will be shown later that at the time when egg-strings are about to be produced the

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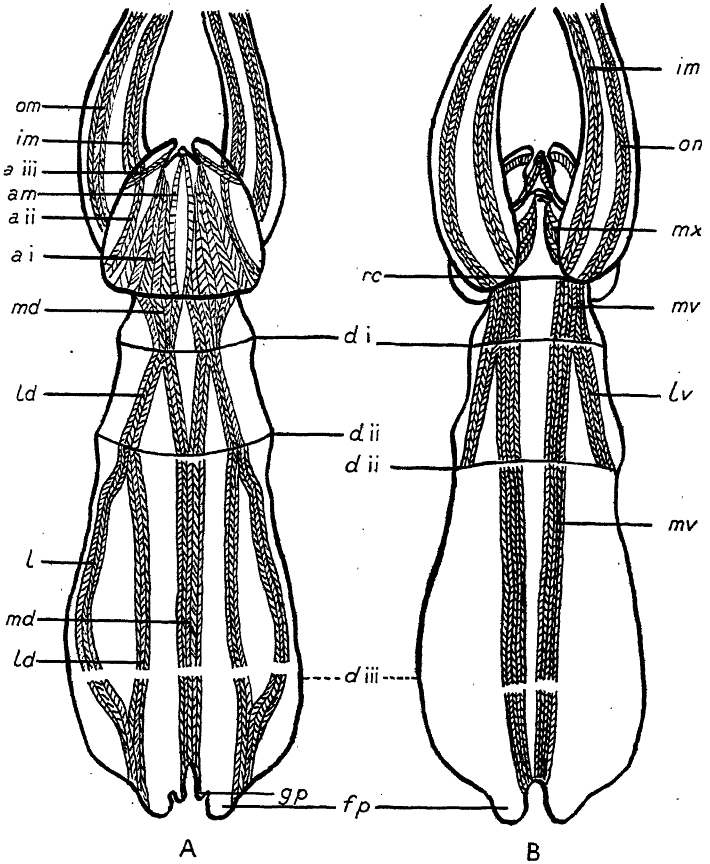
[1] Mr. M. G. L. Perkins has suggested to me that both the swelling of the appendages and the increase in size of the contained glands is merely an oedematous condition induced by the increased pressure within the trunk following upon the increase in size of the ovaries.

TEXT-FIG. 2.



A. Dorsal and lateral muscles. B. Ventral muscles. *a i* to *a iii*, antennary muscles; *am*, muscles of mouth-cone; *d i* to *d iii*, divisions between the trunk segments; *fp*, food-reserve process; *gp*, genital process; *im*, inner maxillipedal muscles; *l*, lateral muscle; *ld*, lateral dorsal muscle; *lv*, lateral ventral muscle; *md*, main dorsal muscle; *mv*, main ventral muscle; *mx*, maxillary muscle; *om*, outer maxillipedal muscle; *rc*, ring of chitin at junction of trunk and cephalothorax.

alimentary canal is functionless; it is at this time that the glands in the abdominal appendages are most active. It is my suggestion, and since I have been unable to obtain living animals upon which to experiment it must remain merely a suggestion, that the gland in the appendage assists in the rapid digestion of the food reserves which must be proceeding if the animal is to survive this period.

THE MUSCULAR SYSTEM.

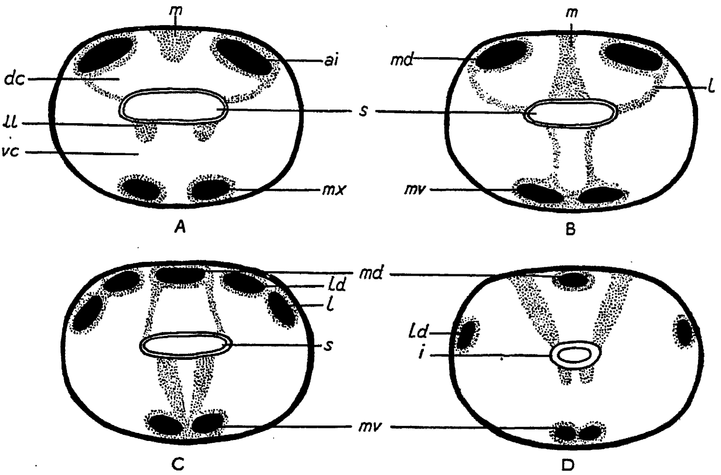
As regards this system the animal is divided into five parts: the cephalothorax and four trunk divisions. The junction of the cephalothorax and trunk is strengthened by an internal ring of chitin; the four divisions of the trunk are marked by slight ridges in the chitin, the two anterior being strengthened with a ring. At all these the muscle-bands become expanded laterally owing to the spreading, not to the increase in number, of the stands, some of which become attached to the chitinous support.

The musculature of the cephalothorax is primarily composed of two large bands of fifty to twenty strands apiece. Of these strands some four-fifths are devoted to the antennae, forming two isosceles triangles which originate on the dorsal 'backfold' of the cephalothorax and run downwards and outwards round the shoulder of the stomach (for whose form see Text-fig. 5) to their insertion on the bases of the first joints of the antennae. Half-way along the outer edges of these triangles two thin bands of muscle (Text-fig. 2 A, *a ii*) curve outward and become attached to the outer edges of the first joints. From the inner sides of the triangles similar narrow bands (Text-fig. 2 A, *am*) run to the mouth cone, being attached, one on each side, near to the tip. Two other bands (Text-fig. 2 A, *a iii*) run diagonally from the tips to about half-way down the sides of the protopodites of the antennae.

The musculature upon the ventral surface of the cephalothorax is principally composed by those of the maxillipedes (Text-fig. 2 B, *im* and *om*) and second maxillae. The maxillipedes contain two main muscles, the outer maxillipedal (*om*) and the inner maxillipedal (*im*), both of which are on the same level, but are on opposite sides of the appendage. They originate upon the ring of chitin at the junction of the cephalothorax and trunk.

The dorsal trunk-system (Text-fig. 2 A) originates as two thick strands which run from the outer edge of the junction of the cephalothorax and trunk, where they are attached to the chitinous ring, to points about a third of the way across the trunk at the level of the first division (*d i*). At this point the two thick bands each split into two. The medial pair of bands run backwards and inwards to meet in the middle line at the second division (*d ii*). Here they fuse to form the median dorsal muscle (*md*) which runs to the posterior end of the trunk, being broken in the usual manner at the third division (*d iii*). The two latero-dorsal muscles (*ld*) run backwards and outwards as far as the second division, where each gives off a lateral branch (*l*) running backwards and slightly ventrally to form a narrow

TEXT-FIG. 3.



Diagrammatic sections to show the body-cavities. A. Posterior region of cephalothorax. A. Anterior region of the trunk. C. Middle region of the trunk. D. Region of the intestine. *i*, intestine; *s*, stomach; *vc*, ventral chamber; *dc*, dorsal chamber; *l*, lateral chamber; *m*, median partition; *ll*, origin of ventral partitions. Other lettering as in Text-fig. 2.

band along the outermost edge of the body. Both the lateral and the latero-dorsal muscles are broken at the third division, between which division and the outer edge of the food-reserve process (*fp*) they unite prior to attachment to the latter.

The ventral trunk-system (Text-fig. 2 B) originates as two main ventral muscles (*mv*) inserted upon the ventral side of the chitinous ring, which split, shortly before reaching the second division, into two latero-ventral muscles (*lv*); these run only as far as the second division, where they terminate upon the bar of chitin. The two main ventral muscles run parallel to each

TEXT-FIG. 4.

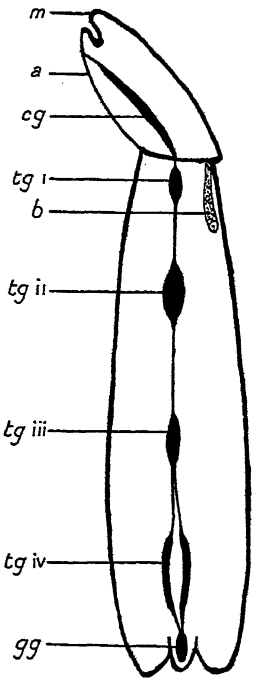


Diagram of the glandular system as seen from the left side. *a*, antenna; *b*, bromatophore; *cg*, cephalothoracic gland; *g*, gland in the genital process; *m*, mouth-cone; *tg i* to *tg iv*, trunk-glands.

other immediately on either side of the median line, being broken at the usual divisions, to the end of the trunk.

There are also five pairs of slender muscles directly connecting the ventral and dorsal body-walls of the trunk. These muscles are at equal distances from each other and from the lateral walls of the trunk. In parasites in the first sexual phase they pass through the ovary. Their possible action is to control the swelling action of the trunk under the growth of the ovaries.

THE BODY-CAVITIES.

Wilson (1915) states that 'there is no heart nor any blood vessels in the Lernaeopodidae; there are not even any lacunae through which the blood might flow. The body is virtually a bag, in the cavity of which the various organs are suspended, while the spaces around them are entirely free, and every part is in direct communication with every other part'. In Lernaeopoda this is only true as regards the anterior third of the cephalothorax, and in roughly that portion of the abdomen which is included in the lower half of Text-fig. 11. The remainder of the body is divided by longitudinal partitions. I hesitate to term these mesenteries (even though they do support the alimentary canal) for two reasons. Firstly these partitions are amazingly thick in section; secondly they are outgrowths of the epimysia of the muscles and seem to be more closely connected with these latter than with the alimentary canal.

If we examine a section through the cephalothorax just behind the opening of the oesophagus into the alimentary canal we find that there are two partitions each attached to the most lateral edges of the antennary muscles. The body is at this point therefore divided into almost equal dorsal and ventral chambers.

Taking next a section through the posterior region of the cephalothorax (Text-fig. 3 A) we find that the antennary muscles (*ai*) are in a more dorsal position so that the ventral chamber (*vc*) is far larger than is the dorsal (*dc*). Between the two muscles a patch of connective tissue (*m*) is commencing to grow downwards towards the alimentary canal. This partition differs from all the others in being at no point directly attached to a muscle, and in the fact that it only extends for five or six millimetres. A couple of lobes of connective tissue (*ll*) appear on the ventral wall of the stomach opposite to the maxillary muscles (*mx*).

In Text-fig. 3 B, a section across the anterior region of the body, we find the body divided into five cavities, three ventral and two dorsal. The antennary muscles have given way to the main dorsals (*md*) and the maxillary to the main ventrals (*mv*). The twin ventral nerves (not shown in the diagram) lie along the central ventral chamber.

In Text-fig. 3 C, a section across the middle of the abdomen, we find the condition which pertains throughout almost the whole length of the abdomen. There is a pair of dorsal partitions coming from the sides of the single main dorsal muscle (*md*) and a pair of ventral partitions coming from between the paired main ventrals (*mv*). The combined thickness of the ventral partitions is considerably greater than that of the space between them. The two great lateral cavities are occupied by the ovaries, but the small dorsal and ventral cavities contain only the body fluid.

As in the anterior end of the body, it is the ventral partitions which break down before the dorsal (Text-fig. 3 D). This diagram represents a section taken through the region of the intestine, and the lateral cavities are occupied by the oviduct and its terminal lobe (vide subter), while the space between the alimentary canal and the main ventral muscle contains the spermotheca. The two dorsal partitions are now attached to the body-wall with the main dorsal muscle lying between them.

Subsequently the dorsal partitions also disappear, so that, as already stated, the most posterior region of all, like the most anterior, is free from divisions of any kind.

THE GLANDULAR SYSTEM.

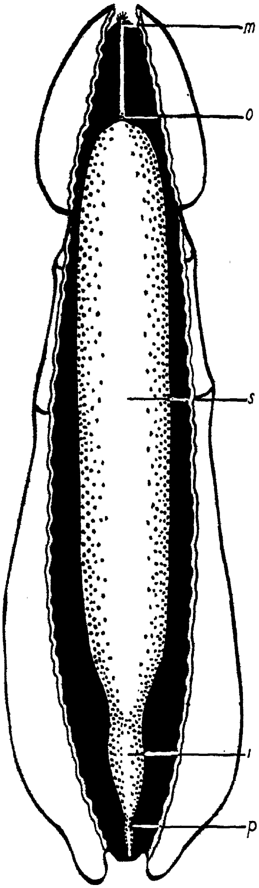
The glands in the mature parasite are of two kinds. The first kind (the bromatophores) I have already dealt with in detail (Parasitology, vol. xviii, no. 4). They are ' ten elongated sacs, five upon each side of the middle line ', occupying ' a position between the junction of the cephalothorax and trunk and the anterior margin of the ovary '. ' Their anterior ends, which are very narrow, open into a pouch whose upper wall is the flap of the cephalothorax and which thus communicates with the surface exactly at the junction of the cephalothorax and trunk. The surface is completely protected by the overlapping of the cephalothorax. These bodies differ in size and shape, but agree in having a blind posterior end more or less clavate, in tapering postero-anteriorly, and in the granular aspect of their walls '. I can find nothing in my sections to cause me to modify either my description or my subsequent suggestions as to the function.

The second set of glands takes the form of two chains running one up each side of the animal and opening upon the antennae (Text-fig. 4). Each chain consists of one elongated cephalothoracic gland (*cg*) and five trunk-glands. The anterior four-trunk-glands (*tg i* to *tg iv*, Text-fig. 4) lie one in each of the segments already marked out by the muscular system. The fourth trunk-gland is split in two, one-half lying on either side of the oviduct. The fifth trunk-gland (*gg*) lies in the genital process. The gland in the abdominal appendage has already been noticed; it is identical in structure with, but it is not connected to, the trunk-glands.

Taking a section across the gland *cg*, we find that the gland at this point is divided into three lobes and each lobe into lobules. Each lobule has a slit-like lumen which becomes round and clearly defined in the anterior half millimetre, being in this region lined by a chitinous invagination of the cuticle. The trunk-glands, although constant in position in different specimens, have no constant number of lobes and lobules. This whole system is linked by fine ducts, each lobe of each gland adding one duct to the main collection. It would therefore be perfectly correct to describe the whole system as a series of ducts running down each side of the animal, each duct being swollen at regular ' nodes ' into a gland, new lobes (and hence more ducts) being added at each ' node '.

In addition to these two chains there are a number of quite irregularly placed gland lobules over the whole of the body-wall, but these have no connexion with each other and there is no means of ascertaining whether they are vestigial or rudimentary.

TEXT-FIG. 5.



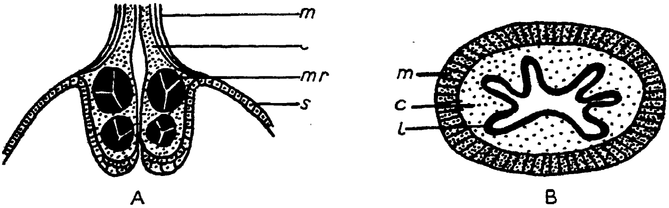
Graphic reconstruction to show the relation of the alimentary canal to the remainder of the body. *m*, mouth; *o*, point where oesophagus passes under the stomach; *s*, stomach; *i*, intestine; *p*, proctodaeum.

The chief interest in this system lies in the segmentation. It is now clearly shown that the trunk consists of the four segments marked out by the muscular system, and in all probability a fifth now forming the genital process, while the two abdominal appendages may well represent the vestiges of a sixth segment.

The Digestive System.

The oesophagus (Text-fig. 5), between 1 and 2 millimetres long and only 20 to 30μ thick, leads from the end of the mouth-cone (*m*) to the stomach (*s*), which it enters (Text-fig. 5, *o*) upon the ventral surface after passing for about a millimetre under the proximal end. In the mouth-cone, and at intervals along the oesophagus, muscles are inserted. Those attached to the mouth-cone have already been noticed in the account of the muscular system. Those inserted along the oesophagus originate at regular intervals along the sides of the mouth-cone. The entire oesophagus is lined by a chitinous invagination of the cuticle (Text-fig. 6 B), and therefore forms the stomodaeum. At the entry of the oesophagus into the stomach there is a valve composed of two circular rings of striped muscle (Text-fig. 6 A, *mr*) embedded in a broad sheath of connective tissue (Text-fig. 6 A, *c*). The stomachic surface (Text-fig. 6 A, *s*) of this valve is lined with transitional epithelium, while the oesophageal surface is, of course, covered by the cuticular invagination.

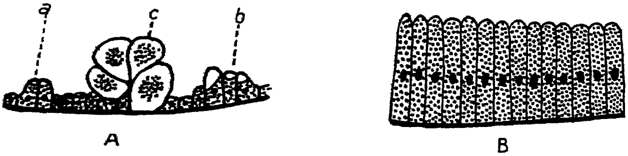
Text-fig. 6.



A. The oesophageal valve as seen in vertical section. B. The oesophagus as seen in transverse section. *c*, layer of loose connective tissue; *m*, longitudinal muscle-layer; *l*, chitinous invagination; *mr*, one of the muscle-rings; *s*, stomach wall.

The stomach (Text-fig. 5, *s*) is normally a wide tube occupying the posterior half of the cephalothorax and the anterior four-fifths of the trunk. That portion of the stomach which immediately surrounds the valve is lined with cubical epithelium, but at frequent though irregular intervals throughout the remainder of the stomach cones of columnar epithelium are found whose entire surface is covered with ovoid bodies, the ‘ gland-cells ’ of Wilson. These cells, however, are not permanent glands, but are constantly being formed and broken down in the following manner: one of the columnar cells, apparently identical with all the others, increases in size (Text-fig. 7 A, *a*) and becomes vacuolated (Text-fig. 7 A, *b*). These vacuoles increase in number and size, their content becoming granular until the protoplasm is reduced to a semilunar mass at the base of the cell (Text-fig. 7 A, *c*). Either of two courses may now be followed. The cell may burst and the pellet of granules be discharged, a new outer cell-wall presumably being formed by the residual protoplasm; or the entire cell may become detached. I have frequently found both free cells and discharged pellets in the same region of the stomach.

TEXT-FIG. 7.

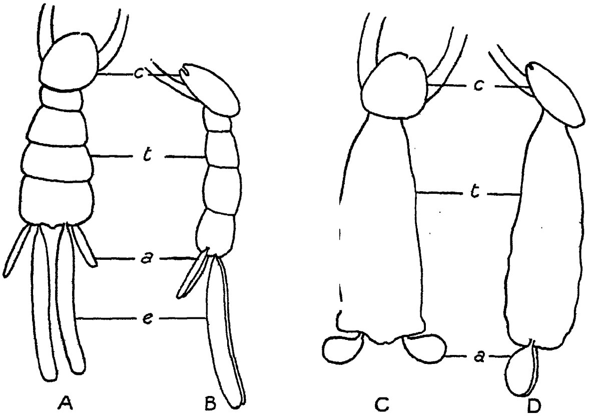


A. A portion of the stomach wall seen in transverse section. B. Columnar cells of the intestinal tract seen in transverse section. *a*, *b*, and *c*, stages in the vacuolation of the stomachic cells.

The intestine (Text-fig. 5, *i*), macroscopically differentiated from the stomach by a slight constriction, occupies the next two millimetres of the canal. This region is lined with columnar epithelium (Text-fig. 7 B) which varies in height in a regular manner so that the opened and flattened intestine appears papillate. For the last half millimetre the papillae are long and interlaced so that it would be impossible for any solid excrement to pass; a decrease in size then follows, the epithelium becoming cubical and finally covered by an invagination of the cuticle forming the true proctodaeum (Text-fig. 5, *p*). The actual vent is formed by the canal widening dorso-ventrally and narrowing laterally till the anus and the groove dividing the two genital processes become one and the same thing.

It may be argued from this that the course of nutrition is probably as follows. The muscles attached to the oesophagus and mouth-cone contract and by doing so increase the capacity of the oesophagus. This creates a sucking action which draws blood from the mass of rasped muscle fragments with which the action of the spiny pads on the maxillipedes has surrounded the mouth; the fringe of hairs on the latter ensure that only blood should pass. The oesophageal valve is now opened and the food carried into the stomach by peristaltic movement of the oesophagus. That the food can only be blood is indicated as much by the small size of the oesophagus as by the fact that no recognizable food-stuff occurs in any part of the intestine. If the muscle itself were the food we should expect to find traces of it at least in the anterior portion of the stomach; but even in those specimens whose head was completely embedded in muscle raspings I have found the canal free from any solid content.

TEXT-FIG. 8.

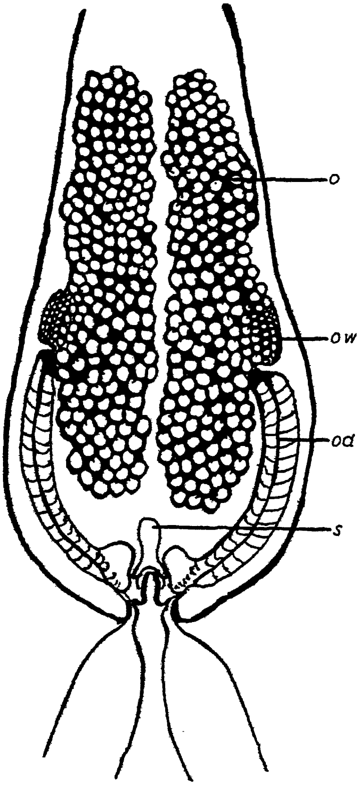


Body-form in first and second sexual phases. A. Dorsal aspect in the second sexual phase. B. Lateral aspect in the second sexual phase. C. Dorsal aspect in the first sexual phase. D. Lateral aspect in the first sexual phase. *a*, abdominal appendages; *c*, cephalothorax; *e*, egg-strings; *t*, trunk.

THE REPRODUCTIVE SYSTEM.

The life of the sexually mature female Lernaeopoda is divided into three phases; I am not aware for how long each of these phases lasts. They are:

TEXT-FIG. 9.



Graphic reconstruction of the reproductive system. *o*, ovary; *od*, oviduct; *ow*, germinal epithelium; *s*, spermatheca.

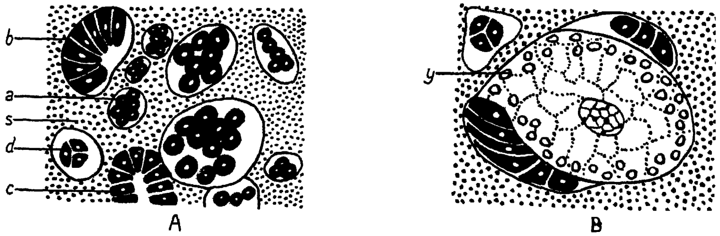
(i) 'About to produce egg-strings' (Text-fig. 8 C and D). The trunk is much swollen, being almost cylindrical in section; the 'abdominal appendages' are swollen into pear-shaped sacs held stiffly out from the body.

(ii) ‘ Having just produced egg-strings ’ (Text-fig. 8 A and B). The trunk is flattened dorso-ventrally and clearly segmented. The outline of the alimentary canal may sometimes be seen through the body-wall. The ‘ abdominal appendages ’ are thin, tapering strings hanging limply from the body.

(iii) A period intermediate between these, in which the trunk is oval in section, egg-strings are carried, and the specimen may or may not carry a male. The abdominal appendages are cylindrical.

Of twenty-five specimens taken within a week of each other from Scylliorhynus (Scyllium) canicula at Plymouth, two were in the first stage, six were in the second, and the remainder were in the third. These three stages are so marked and distinct that I would have regarded them as separate species, had not a microscopic examination of the appendages convinced me that this were not the case.

TEXT-FIG. 10.



A. Tangential section through the germinal epithelium. B. An ovum in which the yolk is just beginning to appear seen in transverse section. Both from photographs. *a*, *b*, and *c*, stages in the development of an egg-string ; *d*, a formal string seen in transverse section ; *s*, stroma of ovary ; *y*, yolk-globule.

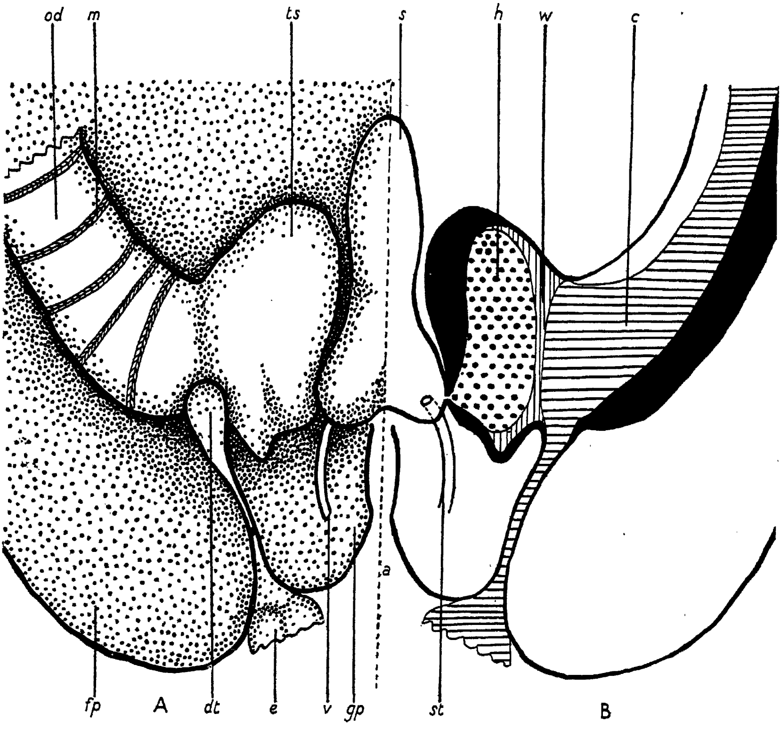
The organs of reproduction (Text-figs. 9 and 11) consist of paired ovaries (*o*) and oviducts (*od*), with a median spermatheca (*s*) into which lead paired sperm-tubes (*st*) from the vulvae (*v*).

The ovaries vary in size from cigar-shaped masses, occupying about one-third the length and one-sixth the width of the trunk of a virgin female, to irregularly shaped masses, occupying the entire trunk and crushing the alimentary canal to a ribbon between them, in the case of a parasite in what I have described as the first sexual phase. Microscopically, the ovary is divided into three zones. The first zone is the germinal epithelium. In the young it covers the entire surface; it does not, however, grow with the ovary, but remains in adult specimens only as an ovoid patch on the lateral periphery of the ovary. From this patch a cell is given off which passes into the stroma where it lies quite freely in a vacuole. This cell has a small spherical nucleus and a thin cell-wall; the periphery of the vacuole in which it lies is denser than the surrounding matrix, and thus forms a 'pseudo-membrane' which may be easily mistaken for the cell-wall of the oogonium. This latter multiplies rapidly, at the same time migrating inwards, till a loosely knit spheroid mass of cells comes to lie in the vacuole (Text-fig. 10 A, *a*). This terminates the 'zone of production' which therefore comes to occupy an ellipsoid patch on that portion of the periphery of the adult ovary which lies nearest the lateral body-wall.

The next zone, occupying the remainder of what Wilson terms the ovary (Text-fig. 9, *ow*), I should like to call the zone of formation. Here the numerous cells in the ovary unite to form a string either two or three cells thick and proportionately long (Text-fig. 10 A, *b* and *c*). By the time that this string has been formed it will have been forced inwards (by the growth of new material) to a position alongside the alimentary canal. Any further growth can therefore only be in an anterior or posterior direction. About this time the terminal cell of each string commences to grow very rapidly until it attains a diameter of about 70μ, when yolk-globules begin to appear in the protoplasm, mostly towards the periphery of the cell (Text-fig. 10 B). This figure (semi-diagrammatized from a photograph) is not quite through the plane of the 'tail' of cells which is cut obliquely in two places. The yolk-globules (*y*) increase in number and in size till all that remains visible of the original protoplasm is a small area surrounding the nucleus. The mature ovum is a sphere of some 150μ to 200μ in diameter with a tail of cells wrapped round its circumference.

The oviducal walls are modified into a cement-gland. In the upper portion this modification affects only that half of the duct which lies nearest the lateral body-wall, the modified portion being crescentic in section (Text-fig. 12, *od*). At the posterior end the duct turns sharply towards the middle and ends blindly in a rounded sac (Text-fig. 11, *ts*). This sac is joined to the spermatheca by a narrow connecting tube; the wall of the sac

TEXT-FIG. 11.



Graphic reconstruction of posterior end of trunk to show the ducts of the reproductive system. A. Left side. Surface view. B. Right side. Diagrammatic frontal section. *a*, anus; *c*, unchanged cement; *dt*, dorsal tube; *e*, egg-string; *gp*, genital process; *h*, hardening fluid; *m*, muscle-band on the oviduct; *fp*, food-reserve process; *gp*, genital process; *od*, oviduct; *s*, spermatheca; *st*, sperm-tube; *ts*, terminal sac; *v*, vulva; *w*, partition of hardened cement.

may be fused with that of the spermatheca in old specimens (as in that from which the reconstruction was taken) leaving only a narrow pore. The glandular modification of the wall stops before the inward turn, but there is a modification of the wall of the terminal sac in that portion which lies nearest to the spermatheca. These two glandular patches secrete chemically different substances. The long patch secretes the cement proper (*c*), while the sac is filled with a chromophobic substance (*h*) which appears to react with the cement to give further substance (*w*), probably solid, and at any rate impermeable to the 'hardening' substance.

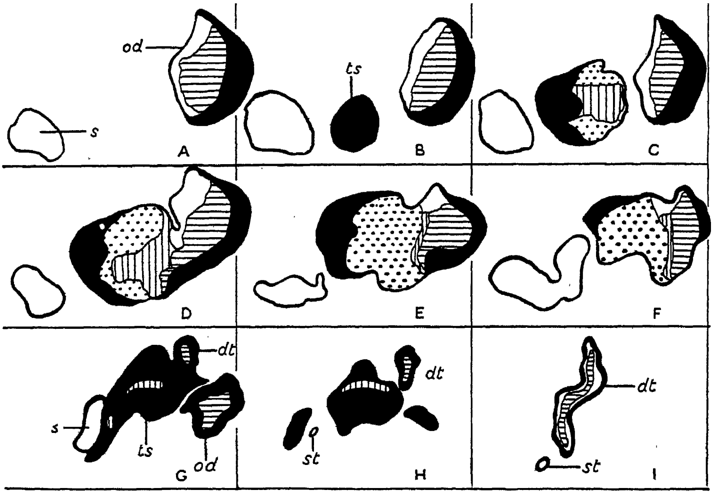
From the junction of the duct and its terminal sac a tube (*t*) is given off from the dorsal surface, which bends sharply in a posterior direction, and finally becomes merged with the fold that separates the genital and food-reserve processes.

The walls of the oviduct are ringed at intervals with bands of striped muscle (*m*).

The details of the lower portion of the reproductive system are best understood from Text-figs. 11 and 12. Text-fig. 11 A is the dorsal view of this part in a parasite in the third sexual phase; Text-fig. 11 B is a diagrammatic frontal section, presuming all parts to be in the same plane, while Text-fig. 11 A is a graphic reconstruction. *od* is the oviduct terminating in the sac (*ts*). This is connected by a tube to the spermatheca (*sp*), which is bifurcate posteriorly, each arm being connected through a sperm-tube (*st*) to the exterior through the vulvae (*v*). The dorsal tube (*dt*) runs into the slits between the folds as already stated. Text-fig. 12 is a series of diagrammatized camera lucida drawings through the region of the right-hand side of Text-fig. 11. Black represents the walls of the tube, dots the 'hardening fluid', horizontal lines the normal cement and vertical lines the cement after the action of the hardening fluid. A shows only the spermatheca and the oviduct. In B the top of the terminal sac has just been cut, while C shows a portion of the hardened cement surrounded by the hardening fluid. D, E, and F show the changes which occur in the shape of the spermatheca, and in them is seen the impermeable barrier of hardened cement. G, H, and I show the relations of the various ducts, the lettering being identical with that employed in Text-fig. 11.

Let us suppose that the animal is about to produce egg-strings. The old egg-strings have been shed ; the body is distended to bursting with mature ova. The walls of the oviduct and its terminal sac are in a state of active secretion, and the oviduct is filled with cement which is protected from the action of the hardening fluid by the partition of hardened material (Text-fig. 11, *w*). Spermatozoa have passed down the connecting tube and are floating in the fluid. A mature egg enters the upper part of the oviduct, there is a rhythmic contraction of the muscular bands, and the egg passes downward driving before it a wave of cement. The partition of hardened material presents more resistance to the cement than does the open dorsal tube, down which the cement accordingly passes : so does not the egg. This is many times the size of the dorsal opening and breaks

TEXT-FIG. 12.



Diagrammatized camera lucida drawings of sections from which Text-fig. 11 B was reconstructed, showing the relations of the hardened and unchanged cements and of the ducts. Lettering as in Text-fig. 11.

down the partition passing into the sac carrying on it a thin coating of cement. Before the fluid has had time to take effect the egg becomes fertilized. The sac contracts and the egg is driven back along its previous path. But by now another egg has reached the bend and is being forced round it. The fertilized egg, now covered by a shell of hardened cement, has only one possible course ; it enters the dorsal tube and passes out through it to become embedded in the mass of cement which its own descent forced out. Further eggs follow, each pushing before it a small quantity of cement, each having its shell hardened in the terminal sac, and the egg-string begins to take shape. Finally the last mature egg is forced out, the oviduct becomes again filled with cement, the hardened partition is re-formed and the parasite sets to work to refill its newly freed stomach.

Nervous System.

This differs in neither detail nor type from that figured (1910) and described (1915) by Wilson. There is a small supra- and a large sub-oesophageal ganglion from which latter nerves run to the various appendages. The ventral chain is represented by a pair of nerves which run posteriorly within the ventral body-chamber. These show no ganglionic swellings and apparently do not contain ganglion cells. In fact, to quote Wilson (1915), ' the nervous system is practically concentrated into the supra-oesophageal ganglion '.

Conclusion.

In conclusion I should like to point out that I have deliberately omitted all mention of histological detail. In the present condition of the science of invertebrate histology I feel that an account of the histology of a somewhat obscure animal would only add to the general confusion.

I should like to express my deep gratitude to Professor MacBride, F.R.S., both for the opportunity to carry out this work and for the great help and encouragement which he has given me. My thanks are also due to Mr. H. R. Hewer, M.Sc., for many suggestions as to the technique and illustrations.

To my friends Professor S. G. Manvalaramanujam, M.A., Ph.D., Mr. W. H. Leigh-Sharpe, M.Sc., Mr. H. K. Mookerjee, M.Sc., and Mr. W. L. Oakley, I wish to offer my thanks for a host of little suggestions and criticisms which have led to the improvement of this paper.

Dr. Wilson and Dr. Brian have both very kindly sent me copies of their works.

LITERATURE CITED.

For all general information as to the Lernaeopodidae reference should be made to the paper by Wilson (1915).

Claus, Karl (1862).—" Ueber den Bau und die Entwickelung von Actheres pecarum ", ' Zeit. wiss. Zool.', vol. 11.

Fasten, N. (1914).—" Fertilisation in the parasitic copepod Lernaeopoda edwarsii ", ' Biol. Bull.', vol. 27.

Gray, Peter (1926).—" On the nutrition of the male of Lernaeopoda scyllicola ", ' Parasitology ', vol. xviii.

Kurz (1877).—" Studien über die Lernaeopodiden ", ' Zeit. wiss. Zool.'

Leigh-Sharpe, W. H. (1916).—" Lernaeopoda scyllicola n. sp. a parasitic copepod of Scyllium canicula ", ' Parasitology ', vol. viii.

Wilson, C. B. (1910).—" Development of Actheres amblopltis Kellicott ", ' Proc. U.S. Nat. Mus.', vol. 39.

—— (1915).—" North American parasitic copepods belonging to the family Lernaeopodidae, with a revision of the entire family ", ibid., vol. 47.

The Muscles of the Adult Honey-Bee (Apis mellifera L.).

By

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North of Scotland College of Agriculture, Aberdeen.

With 1 Text-figure.

PART III.

THE HEALTHY MUSCLES OF THE ADULT HONEY-BEE. THE CHEMISTRY OF THE MUSCLES.

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THE CHEMISTRY OF THE MUSCLES.

No physiologist seems to have attempted the detailed chemical analysis of the muscles of any insect. Written knowledge of the chemistry of the muscles of the adult bee seems limited to remarks like Holmgren's (1910) when he discusses the albuminous nature of the sarcosomes of some insects. Strauss (1911) gives some interesting records of analysis of adult workers and drones, but as whole insects were analysed for glycogen, fat, and nitrogen, the figures cannot be applied to muscles alone.

Aronssohn (1911) analysed the ashes of drones for the elements which remained after the dessication of the insects. Since he does not state that the drones were washed clean from external matter before they were dessicated, I presume that this was not done. As bees may easily be contaminated with external matter, there is the possibility that external matter is included in his tables of the elements. This might explain the presence of aluminium and zinc besides various proportions of the other elements in his tables. Mr. Godden of the Rowett Research Institute kindly discussed with me the results of his own analyses (unpublished) of workers and larvae. He too analysed the ash of unwashed insects. Mr. Duthie carried out some partial organic and inorganic analyses of bees in the Chemistry Department, Marischal College. He did not find copper in the ashes. The equipment of our Entomological Department is inadequate for detailed investigation of the physiological chemistry of the muscles of the bee, so only the bare outline of the subject is attempted in this paper, and all quantitative measurements are omitted. The muscles analysed were very largely fibrous muscle, owing to the difficulty of obtaining tubular muscle sufficient for analysis yet free from other tissue. The thoraces of worker bees were much employed for analysis. In the thorax the chief tissues besides the muscle are the thoracic salivary glands and ducts and the nerves. It was impossible to remove the latter completely, but the former were invariably removed, though I never obtained a reducing sugar reaction from them, but glycogen seems to be present in them sometimes from their staining with iodine. Many of the methods employed were microchemical as described in Abderhalden (1912) and elsewhere, otherwise the plan of analysis is such as described for vertebrate muscles in books of physiological chemistry, e.g. Cole (1926).

pH Values of Muscle.

The hydrogen-ion concentration and its variation in organic substances is often of importance in chemical analysis, but this method of investigation does not seem to have been applied in detail to the tissues and secretions of the bee. Till our knowledge of the physics and chemistry of the muscle of the bee is greatly extended, the value to be attached to pH readings of the muscle can have only a general significance; especially as muscle is an unstable complex of many proteins, inorganic salts, and other substances, each of which would have its own pH values. Mr. Newlands, of the Agricultural Chemistry Department, receives my most cordial thanks for obtaining the pH values of bees' muscles by the method of the quin-hydrone electrode. The thoraces of a number of bees were removed from living insects and crushed in a minimum volume of distilled water to which the hydroquinone was added. The time from when the bees were alive to when the first pH reading of their muscles was taken, was only a few minutes at a room-temperature of about 17° C. The pH of the tissue treated thus was 7·1 at the first reading, but altered to 6·6–6·5 after a few minutes. The latter reading seems the more constant. As Mr. Newlands remarked, these values only indicated that the tissue as a whole was almost neutral in reaction, though it showed quite a wide range in value.

The most important constituents of living striated muscle of vertebrates are: (1) proteins, (2) pigment, (3) fat, (4) nitrogenous extractives, (5) non-nitrogenous extractives, (6) inorganic matter. The chemical composition of the muscles of the bee will be considered under the above headings.

Proteins.

Extracts of both tubular and fibrous muscle as well as portions of the fibres themselves give protein colour-reactions to xanthoproteic, Millon's, biuret, sulphur, and Molisch's tests. The extracts, if freshly prepared, are precipitated by the salts of the heavy metals, the so-called 'alkaloidal' reagents, strong alcohol, and heat.

In the fresh muscle extract prepared from the entire thoraces of 100 bees in 5 per cent. magnesium sulphate during 24 hours in an ice-chest, a coagulation occurs at 55° C. and another at 75° C. The coagulum formed at the respective temperatures may be analogous with that of paramyosinogen and myosinogen which are the chief proteins in living muscles of vertebrates.

Tubular and fibrous muscles after suitable preparation give all the reactions for myosin or the protein of dead muscle of higher animals. The precipitate of myosin dissolved in ammonium sulphate coagulates between 55°–60° C.

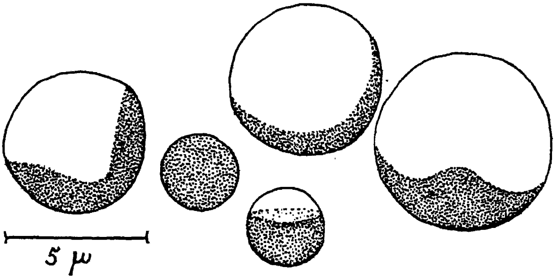
Of the various groups of proteins known I have found in the muscles of the bee albumins and globulins. Albuminoids or scleroproteins perhaps are present in some of the tendinous attachments of muscle-fibres. Phosphorous is found in the muscles, but as it may be combined in various ways it does not prove the existence of phosphoproteins. Under conjugated proteins comes the chromoprotein containing cytochrome (cf. Keilin, 1925–6). The pigment is probably distributed throughout muscle-fibrils and sarcosomes of fibrous muscle, and from Keilin's records of its wide dispersal in the animal kingdom it seems plausible that it would be found in tubular muscle is sought for carefully.

The glycoproteins appear as albumen plus carbohydrate group in the sarcosomes. Nucleoproteins are probably present owing to the large number of nuclei in muscle-fibres, but I have no certain chemical evidence for their existence. Hydrolysed proteins can be obtained from the native proteins by suitable treatment.

During the analysis of fibrous muscle it is impossible to get rid of the sarcosomes, on the other hand it is possible to dissect free from muscle-fibrils sufficiently large quantities of sarcosomes for analysis either on a microscope slide or in a test-tube. Of course, however rapidly and frigidly the dissection is accomplished, the mixture of sarcosomes and collecting fluid is liable to contain blood-plasma and soluble extractives of the muscles. The sarcosomes, when isolated in distilled water, become spherical (Text-fig. 1) with a shallow cup of protoplasm at one pole and a very delicate wall for the rest of the sphere. The interior of the sphere is occupied by a fluid. They show various modifications in shape in different fluids (cf. Knoche, 1909; Regaud et Favre, 1909; Bullard, 1912, who describe them for various animals). However, it seems to me that their change of shape is explicable as the reactions of conjugated protein to different environments.

The sarcosomes give all the above-mentioned reactions for proteins, including a reddish violet colour to a modification of Adamkiewicz's reaction which probably indicates that they possess an indole or skatole radicle in the molecule of one of their proteins. Molisch's reaction indicates that a protein is combined to a carbohydrate group.

Text-fig. 1.



Some of the shapes of sarcosomes from living fibrous muscle dissected in distilled water.

Pigment.

All the tubular muscles when alive are colourless by reflected and transmitted light. The fibrous muscles in the resting-middle-aged bee of the three castes vary slightly in colour from a pale yellow to a pale orange tinge. The tint is the same for all the muscles of an individual bee. The range in colour does not seem to be a chromatolysis due to fatigue, though I have not been able to tackle the problem of correlation of colour and fatigue. All the progeny of a queen seem constant in the colour of their muscles. Orange-tinted muscles are commoner in black than in yellow bees, but there is not a precise correspondence between the colour of the integument and muscles. The colour is not seen in the muscles of the pupa or of the very young bee in which the muscles are whitish, but from Keilin's work on the blow-fly it seems plausible that the pigment cytochrome would be present in the immature stages of the bee. The colour develops gradually apparently with the hardening of the integument, and it persists until death and afterwards for a variable period depending on the environment of the muscle. In strong sodium or potassium hydroxide fibrous muscles turn orange red, as Keilin remarked. A yellowish brown colour is seen in the filtrate obtained after crushing fibrous muscle in water, and this colour persists after long boiling of the filtrate. It is apparently due to iron bound in protein which is not coagulated by heat till a trace of ammonium sulphate is added to the solution. The protein coagulum is brownish and it turns bright blue when tested for the Prussian-blue reaction of iron. The filtrate, after the removal of protein coagulum, is usually colourless, but it may contain traces of iron.

The colour of the living and freshly-examined fibrous muscle is due to the respiratory pigment cytochrome lately described by Keilin (1925, 1926). According to Keilin, cytochrome is very easily oxidized. It has the properties of a thermostable peroxidase and when oxidized has all the characters of a parahaematin compound. In a reduced state it has a very characteristic absorption spectrum composed of four main bands, and it is the only colour seen spectroscopically. When it is oxidized, the bands disappear, and its state, as seen spectroscopically, denotes only the difference between the rates of its oxidation and reduction.

Through the help of my friend Mr. Griffith of the Physics Department, we were enabled to see the four absorption bands of cytochrome approximately in the positions described by Keilin. The bands disappeared with oxidation of the tissue. Our apparatus was a microscope fitted with a spectroscopic prism. Mr. Griffith also receives my warmest thanks for his help in fitting up apparatus for the examination of muscle by polarized light.

Fat.

In discussing the tracheation of fibrous muscle (Morison, 1928) it was mentioned that very minute quantities of neutral fat were sometimes found adjoining the smaller tracheae amongst the fibres. The fat is not very intimately connected with the muscle-tissue, yet it is the only fat which I have found with fibrous muscle, whilst tubular muscle seems to lack this type of fat completely. I have not been able to employ methods for the extraction and subsequent estimation of fats and allied substances, but a great variety of staining methods demonstrated no fat.

The lipins will be included under the heading ' fat '. Some observers have drawn a certain amount of evidence for the presence of lipins in sarcosomes and other tissue from the degree of staining with haematoxylin. Macallum's use of haematoxylin as a means of detecting iron, and Gutstein's paper (1926) on the theory of haematoxylin staining, show that very little weight can be attached to such evidence. I am greatly indebted to Professor Cruickshank of the Bacteriological Department for the following method of demonstrating the substance lecithin: the stain, light green, is normally insoluble in ether, but passes into solution in the presence of lecithin. The thoraces of a number of bees were opened up and extracted in ether for two days, then the ethereal extract was poured on to an aqueous solution of light green which coloured the extract. Lecithin in the vertebrates is derived largely from nervous tissue, so this source must be considered in the bee.

Nitrogenous Extractives.

These substances were not investigated in the muscles of the bee.

Non-nitrogenous Extractives.

Glycogen was not found in any of the muscles of the bee, by Best's carmine method of staining or by any other chemical methods attempted. Its place is presumably occupied by a monosaccharide, apparently glucose.

It was stated that both muscles and sarcosomes gave a positive reaction to Molisch's test which indicates that a protein is linked to a carbohydrate group. By rapidly dissecting the thoraces of twenty to forty bees in 10–20 c.c. cold distilled water, a fluid is obtained which is opalescent to reflected light, but cloudy and tinged faintly brownish by transmitted light. The opalescence and cloudiness are due to the protein—chiefly sarcosomes in suspension—and the brown colour presumably to cytochrome or at least the iron found in it. The bees must be freshly killed, quite clean externally, and their heads and post-abdomens should be plucked off with delicate forceps so that no food should escape into the oesophagus lying in the thoracic region. During the dissection the thoracic salivary glands and the portion of the oesophagus are removed. After decanting, centrifuging, and filtering to remove the muscle-fibres and chitin, the opalescent fluid is boiled till all the protein has coagulated, and the remaining fluid is quite transparent and does not give a protein reaction to biuret. Usually it will be necessary to add more water before all the protein has coagulated. If the transparent fluid is tested by Fehling's or Cole's reactions for reducing sugars, it will give a bright orange precipitate of cuprous oxide with the former and a yellow precipitate with the latter. The sugar seems to be glucose. Its osazone with phenyl-hydrazin-hydrochloride is in the shape of yellow, acicular crystals arranged in sheaves or other patterns like those of glucosazone. It seems reasonable to assume that the sugar has been derived largely from the sarcosomes. If the bees used in the experiment have been dead for a day or longer at room temperature, or if the muscles are left soaking for some hours, various chemical changes occur in the tissue with the result that the sugar cannot be demonstrated and that protein is in solution and can only be removed by heat coagulation after the addition of ammonium sulphate.

The extraction of sarcolactic acid was not attempted.

Inorganic Matter.

I cannot give any quantitative measurements of the water content of muscles, nor can I describe the salts except to write that variously shaped microscopic crystals will be formed in glycerine if unfixed muscles are mounted in a mixture of distilled water and glycerine. The salts would be dissolved in the muscle and in the water, but as the latter evaporates and more pure glycerine is added to the mount the salts become visible as crystals unless they are soluble in glycerine.

The elements C, O, N, H, S are present in muscle as organic matter. Iron is easily demonstrable in fibrous muscle, less so in tubular muscle, but very clearly in many of the glandular regions. Muttkowski (1921) has stated that copper is present in the blood of the bee. This leads one to think that it would be found in the tissues, but I have not found a trace of it anywhere in the bee either by methods of staining or by a colorimetric test after reducing the tissue of fifty bees to ash. Mr. Welch, of the Agricultural Chemistry Department, also could not find copper in the ash of fifty bees when he used another very delicate colorimetric test. Aronssohn (1911) found a very small percentage of copper in the ash. Phosphorus would be found in lecithin. It may occur in tissues in the inorganic form of phosphates and in various complex organic combinations. My experiments on the whole show that it exists in the bee, but they are too long and inconclusive to be worth recording. Chlorine is the most likely of the halogens to appear in muscle. It may be present as a salt, but I can offer no definite proof of its existence in the muscles of the bee, and the same applies to the elements magnesium, potassium, and sodium. These elements have been found in the ashes of bees.

THE APPEARANCE OF MUSCLE-FIBRES UNDER POLARIZED LIGHT.

The appearances of muscle-fibres under polarized light have not been studied in detail by me, since an adequate explanation of all such appearances seems to delve too deeply into the physics and chemistry of the muscle of the insect to be possible in the present state of our knowledge. A reader desirous of pursuing this subject should consult the papers of Engelmann (1873), Merkel (1873), and Van Gehuchten (1886), all of whom examined the muscles of the bee during their investigations. Schaeffer (1873), Engelmann (1878), Hürthle (1909), Ebner

(1918), and Jordan (1920) discuss in more or less detail the appearances of insect muscle under polarized light. Finally Stübel (1923) has written the most scientific and stimulating paper on the phenomenon as observed in the muscles of the frog.

My observations may be summarized thus : both tubular and fibrous muscle in the bee show isotropic (not illuminated) and anisotropic (illuminated) discs when examined by polarized light with crossed Nicols. The tissue is best examined fresh in water or salt solution, but two-year-old glycerine mounts of fibres fixed in alcohol and variously stained will often show equally good contrast. Muscles fixed, stained, and mounted in a medium like Canadian balsam do not respond to polarized light. Whether fresh or fixed tissue is examined, not all the fibres will show equal degrees of contrast—some will be brilliant with the Nicols crossed whilst others are invisible and the degree of brightness does not depend upon the state of contraction of the fibre. The alary and somatic muscles show strong contrast between isotropic and anisotropic transverse discs and a more obscure longitudinal striation corresponding to their individual fibrils. The cardiac, ventral diaphragm, and alimentary canal muscles are obscure, the fibrous muscle-fibres are bright and can be resolved into their component fibrils, but the latter when separated are too delicate to be distinguishable. Sarcosomes are invisible when isolated and they cannot be determined in the fibre. Selenite plates may be used with the Nicols so as to colour the anisotropic discs, but they do not seem to be an advantage. The tube of the tubular fibre is of some importance optically.

Anisotropy and the depth to which tissue will stain are two essentially distinct phenomena, but they are often associated in a fibre in such a way that the darkly stained discs appear anisotropic and the lightly stained discs isotropic, and when there is a reversal of the striation during contraction there may or may not be a corresponding displacement of anistropic and isotropic discs. The telophragmata of fresh and fixed tubular fibres are usually in the middle of a broad isotropic band, whilst in fibrous fibres they are often seen as dark transverse lines.

Effect of Age and Fatigue on the Muscle.

The effect of age and fatigue on muscle is very difficult to judge and requires greater detail of technique than I have been able to bestow on the investigation. If examined by the ordinary methods, neither tubular nor fibrous muscle seems to show any variation from the normal (as described, Morison, 1927, 1928) as the effect of age or fatigue. However, with fatigue (cf. Holmgren, 1908, 1910) there is probably a difference in colour of fibrous muscle as well as chemical differences, and with age the muscles may show a state in correlation with the division of labour in the colony according to the age of the bee (cf. Rosch, 1925).

Theories of Muscular Contraction.

The cause of the contraction of muscle is still unknown though sought actively by physiologists who seem to be approaching close to the object of their search. Amongst the number of the older workers who in their quest paid attention to insect muscle, the names of Engelmann, Kölliker (1888), Schaeffer, Merkel (1881), and van Gehuchten (1886) may be mentioned. Engelmann's theory was that a muscle-fibril had no vital power of movement, but that if it were warmed it absorbed water from the plasma of the muscle-cell surrounding it. The absorption of water by the fibrils caused them to become thicker and shorter so that the whole muscle contracted. The plasma of the muscle-cells was supposed to contain a secretion giving heat when combined with oxygen and the motor-nerve impulse was supposed to cause oxygen to unite with the substance. Schaeffer supposed that under certain physico-chemical laws the isotropic substance flowed into a series of channels in the anisotropic substance, thereby swelling and shortening the latter. Merkel (1881) reviews the various theories up to date. Van Gehuchten (1886) considered that the longitudinal strands of a regular intracellular network were responsible for the contraction of muscle-fibres. Marshall (1888 and later), working on the muscles of higher animals, held van Gehuchten's views essentially, and he stressed the fact that the nerve-fibres seemed to end in contact with the longitudinal bars of the muscle-fibre.

During later years physiologists have paid very little attention to insect muscle. Modern theories of contraction are founded very greatly on analysis and experimental study of muscles of a more convenient largeness than found in most insects.

In 1907 Fletcher and Hopkins found that lactic acid accumulated in the frog's muscle deprived of oxygen. It had been known previously that lactic acid was present in fatigued muscles, but the work of these two experimenters opened up paths for research along which modern workers are treading.

Many physiologists seem to favour the view that the liberation of lactic acid in the muscle is the cause of contraction. The acid is supposed to be generated and to produce contraction along the fibre at certain intervals which presumably coincide with the transverse striation. The duration of the contraction is very brief, and the lactic acid diffuses away and is neutralized by the numerous buffers of the vicinity. The muscle will be able to contract repeatedly for a short period of time, even in the complete absence of oxygen, but finally the accumulation of acid or the loss of an essential food constituent will result in a condition resembling rigor.

The primary fuel of contraction is a carbohydrate (probably glycogen in the higher animals, perhaps glucose in the bee) and fat, and presumably protein may be used, should the carbohydrate reserve become exhausted. During contraction the temperature rises, and with the onset of fatigue lactic acid appears and the carbohydrate disappears, whilst imperfectly known changes occur in the phosphorous content of the muscle. Oxygen is necessary to restore the muscle completely after contraction. In its presence lactic acid disappears, carbohydrate reappears, carbon dioxide is produced, heat is lost, and the muscle becomes ready to contract again. For a full discussion of the problem the reader is referred to Hill (1926) and to the numerous papers of his colleagues. Meyerhof (1924) and Harden (1926) should also be consulted. Warburg's theory of the role of iron in tissue respiration is of particular interest, considering the distribution of this metal in the bee.

Theories to explain how the liberated lactic acid produces contraction of the tissue may be called the surface-tension theory, the osmotic theory, the swelling theory, and the theory of liquid-crystals. Hill (1925) gives reasons for regarding the surface-tension hypothesis of muscular contraction in its simple form as untenable. The osmotic theory requires a semipermeable membrane to enclose each ultimate contractile element, whilst the lactic acid is formed within the membrane and attracts fluid of higher molecular weight and thereby produces a shortening of the element. The swelling theory supposes that contraction is due to the colloids of the muscle-cell absorbing water during the production of lactic (and phosphoric) acid and that relaxation of the muscle is due to a reversal of the process. Embden and his school tend to hold this theory. The theory of liquid crystals was expounded by Garner (1925), who suggested that the tension generated on applying a stimulus to muscle-fibre is due to the formation of a solid film of liquid crystals on the surface of the ultimate fibrils of the muscle. The film is supposed to lie in or upon the anisotropic substance, and the lactic acid is imagined as altering the arrangement of the crystals and so producing contraction or relaxation. Finally Clark (1927) bases a theory of contraction on X-ray diffraction patterns from relaxed and contracted fibres. She considers that the substance in the anisotropic state passes abruptly from the liquid crystal to the solid crystal form, as a result of the increase in activity due to lactic-acid formation.

SUMMARY.

1. The chemistry of the muscles is described in outline. With it are included pH values and pigment.

2. The appearance of muscle under polarized light is discussed.

3. The effects of age and fatigue on muscle could not be determined by the techniques adopted.

4. The theories of muscular contraction are summarized briefly.

BIBLIOGRAPHY.

Abderhalden, E. v. (1912).—'Handb. d. Biochem. Arbeitsmethoden', Bd. 5. Urban und Schwarzenberg, Berlin.

Aronssohn, F. (1911).—" Sur la composition minérale de l'abeille ", ' C. R. Acad. Sci. Paris ', 152, pp. 1183–4.

Bullard, H. H. (1912).—" On the interstitial granules and fat droplets of striated muscle ", ' Amer. Journ. Anat.', 14, pp. 1–46, 7 figs.

Clark, Janet H. (1927).—" A theory of muscle contraction with X-ray diffraction patterns from relaxed and contracted muscles ", ' Amer. Journ. Physiol.', 82, pp. 181–94, 4 figs.

Cole, S. W. (1926).—' Practical Physiological Chemistry ', 7th ed. Heffer, Ltd., Cambridge, England.

Ebner, V. E. v. (1918).—" Über den feineren Bau der Flügelmuskelfasern der Insekten ", ' Sitz-Ber. Ak. Wiss. Wien, Math.-nat. Kl.', Abt. III, Bd. 127, S. 3–32, Taf. I.

Engelmann, T. W. v. (1873).—" Mikroskopische Untersuchungen über die quergestreifte Muskelsubstanz ", Pflüger's ' Archiv f. Physiol.', 7, S. 33–76, Taf. II.

—— (1878).—" Neue Untersuchungen über die mikroskopischen Vorgänge bei der Muskelcontraktion ", ibid., 18, S. 1–25, Taf. I.

Fletcher, W. M., and Gowland, Hopkins F. (1907).—" Lactic Acid in Amphibian Muscle ", ' Journ. Physiol.', 35, pp. 247–309.

Garner, W. E. (1925).—" The Mechanism of Muscular Contraction ", ' Proc. Roy. Soc. London ', Ser. B, 99, pp. 40–56.

Gehuchten, A. van (1886).—" Sur la structure intime de la cellule musculaire striée ", ' La Cellule ', 2, pp. 289–453, Pls. 1–6.

Gutstein, M. (1926).—" Zur Theorie der Hämatoxylinfärbungen. Ein Beitrag zum färberischen Nachweis der Zelllipoide ", Virchow's ' Archiv f. Path. Anat.', 261, S. 846–57, Fig. 1.

Harden, A. (1926).—" The Formation of Lactic Acid in Muscle ", ' Nature ', 118, pp. 895–6.

Hill, A. V. (1925).—"The Surface-Tension Theory of Muscular Contraction ", ' Proc. Roy. Soc. London ', Ser. B, 98, pp. 506–15.

—— (1926).—" The Laws of Muscular Motion (Croonian Lecture) ", ibid., vol. 100, pp. 87–108.

Holmgren, E. (1908).—" Über die Trophospongien der quergestreiften Muskelfasern, nebst Bemerkungen über den allgemeinen Bau dieser Fasern ", ' Archiv f. Mikr. Anat.', 71, S. 165–247, Taf. xiii–xx.

—— (1910).—" Untersuchungen über die morphologisch nachweisbaren stofflichen Umsetzungen der quergestreiften Muskelfasern ", ibid., Bd. 75, S. 240–336, Taf. viii–xiii.

Hürthle, K. (1909).—“ Über die Struktur der quergestreiften Muskelfasern von Hydrophilus im ruhenden und tätigen Zustand ”, Pflüger's ‘ Archiv f. Physiol.’, 126, S. 1–164, Taf. i–viii, 5 Textfiguren.

Jordan, H. E. (1920).—“ Studies on Striped Muscle Structure. VI. The comparative histology of the leg and wing muscles of the wasp, with special reference to the phenomenon of stripe reversal during contraction and to the genetic relation between contraction bands and intercalated discs ”, ‘ Amer. Journ. Anat.’, 27, pp. 1–67, 48 figs.

Keilin, D. (1925).—“ On Cytochrome, a Respiratory Pigment, Common to Animals, Yeast, and Higher Plants ”, ‘ Proc. Roy. Soc. London ’, Ser. B, 98, pp. 312–39.

—— (1926).—“ A Comparative Study of Turacin and Haematin and its Bearing on Cytochrome ”, ibid., vol. 100, pp. 129–51.

Knoche, V. (1909).—“ Über die Struktur der sogenannten ‘ interstitiellen Körner ’ (Koelliker) der Flügelmuskulatur der Insekten ”, ‘ Anat. Anz.’, 34, S. 165–7, 7 Abbd.

Kölliker, A. (1888).—“ Zur Kenntnis der quergestreiften Muskelfasern ”, ‘ Zeits. wiss. Zool.’, 47, S. 689–708, Taf. xiv–xv.

Marshall, C. F. (1888).—“ Observations on the Structure and Distribution of Striped and Unstriped Muscle in the Animal Kingdom, and a Theory of Muscular Contraction ”, ‘ Quart. Journ. Micr. Sci.’, 28, pp. 75–107, Pl. vi.

Merkel, F. (1873).—“ Der quergestreifte Muskel. II. Der Contractionsvorgang im polarisirten Licht ”, ‘ Archiv f. Mikr. Anat.’, 9, S. 293–307, Taf. xv.

—— (1881).—“Ueber die Contraction der gestreiften Muskelfaser ”, ibid., Bd. 19, S. 649–702, Taf. xxx.

Meyerhof, O. (1924).—‘ Chemical Dynamics of Life Phenomena.’ Monographs on Experimental Biology. Lippincott Co., Philadelphia and London.

Morison, G. D. (1927).—“The Muscles of the Adult Honey-bee (Apis mellifera L.). Part I. The Healthy Muscles of the Adult Honey-bee. Somatic Musculature ”, ‘ Quart. Journ. Micr. Sci.’, 71, pp. 395–463, 12 figs.

—— (1928).—“ The Muscles of the Adult Honey-bee (Apis mellifera L.). Part II. The Healthy Muscles of the Adult Honey-bee. Muscles of the Alimentary Canal, Heart, Diaphragms, and the Reproductive Organs, and the Indirect Muscles of the Wings ”, ibid., pp. 563–651, 41 figs.

Muttkowski, R. A. (1921).—“ Studies on the Respiration of Insects. I. The Gases and Respiratory Proteins of Insect Blood ”, ‘ Ann. Ent. Soc. America ’, 14, pp. 150–6.

Regaud, C., et Favre, M. (1909).—“Granulations interstitielles et mitochondries des fibres musculaires striées ”, ‘ C. R. Acad. Sci. Paris ’, 148, pp. 661–4.

Rosch, G. A. (1925).—“ Untersuchungen über die Arbeitsteilung im Bienenstaat. I. Teil: Die Tätigkeiten im normalen Bienenstaate und ihre Beziehungen zum Alter der Arbeitsbienen", 'Zeitschr. f. vergl. Physiol.', 2, Heft 6. (Résumé in 'Biol. Zentralblatt', 46, S. 190–1, 1926.)

Schaeffer, E. A. (1873).—"On the minute Structure of the Leg-muscles of the Water-beetle", 'Phil. Trans. Roy. Soc. London', 163, pp. 429–43, Pl. 33.

Strauss, J. (1911).—"Die chemische Zusammensetzung der Arbeitsbienen und Drohnen während ihrer verschiedenen Entwicklungsstadien", 'Zeitschr. Biol.', 56, S. 347–97, 9 Abbd.

Stübel, H. (1923).—"Die Ursache der Doppelbrechung der quergestreiften Muskelfaser", Pflüger's 'Archiv f. Physiol.', 180, S. 209–49, 4 Abbd.

Arcella. A Study in Cell Physiology.

By

Edward J. Bles, M.A., D.Sc.

With Plates 30–40 and one Text-figure.

With Preface by Prof. F. Gowland Hopkins and Dr. D. Keilin.

PREFACE

The experimental work described in the following paper was carried out, and, in the main, completed during the years 1910–13. But an earnest endeavour to interpret the facts aright, leading gradually to the personal views expressed in the later chapters of the paper, occupied the author's mind almost up to the day of his untimely death in 1926. His work and thought suffered, however, frequent and long interruptions due to serious illness. Had not his activities been grievously inhibited by that failure of health, further experiments suggested from time to time by his maturing thought about his problem would certainly have been made; while the results of his work and the conclusions arrived at would have been published long ago.

Part I of the paper and much of Part II were ready for publication—almost as they stand—in 1914. They describe highly significant experiments admirably contrived and skilfully carried out.

That portion of Part II which deals mainly with theoretical conceptions based on the experimental facts was always felt by the author himself to be incomplete and to call perhaps for modification; but he was never able in his later days to summon strength to deal further with it. It is a matter for profound regret that the paper has to be printed when its author is no longer here to be consulted. The experimental work stands of course upon its own merits. Most of the theoretical discussion was written long enough ago to antedate changes in current terminology; it might have been modified here and there had the author been acquainted with facts quite recently won on related lines. Since the paper was written considerable progress has been made, for instance in the chemical study of isolated oxidase systems. Of this work the author was not fully aware. His discussion of oxidases sometimes displays indeed the working of a mind more accustomed to morphological than to chemical thought.

The editors have thought it well, however, not to interrupt the development of the author's own line of thought by modifications meant to allow for recent changes in outlook. His views are the product of a thoughtful and philosophic mind and he wrote from a strongly personal standpoint. Only he himself could have modified his discussion without the loss of its essential character. While the facts he discovered are highly significant, his expressed thoughts about them are stimulating and provocative. The paper as a whole suggests fertile lines for extended inquiry in a field of profound biological importance.

Certain acknowledgements the author would have wished to be made. First of all those due to his wife whose sympathy was invaluable to so temperamental a worker; Mrs. Bles was closely associated with the whole progress of the experimental work and was able in many ways to assist it. Dr. J. S. Haldane was a source of encouragement from the first. We believe indeed that it was Dr. Haldane's firmly expressed belief in the great biological importance of gas production as observed in Arcella which, more than anything else, urged Bles to his experimental study of the phenomenon. Bles himself always freely acknowledged his debt to Mr. Maxwell, the artist who made the admirable drawings from which the plates now published were produced. The author and the artist closely collaborated and the efforts of the latter never ceased until both were satisfied that things actually seen were accurately rendered.

F. Gowland Hopkins.

D. Keilin.

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PART I

THE GAS-VACUOLES OF ARCELLA DISCOIDES EHRENBERG

CHAPTER I.—THE LITERATURE ON GAS-VACUOLES IN ARCELLA.

The gas-vacuoles of Arcella were first described by Max Perty in 1849. He speaks of them as 'hohlen (bloss mit Luft gefüllten ?) Räumen' (Perty, 1849, p. 124). In a later paper (1852, p. 184) he says: 'Die Rhizopoden, namentlich Amoeben und Arcellen, können willkürlich Vacuolen, Hohlräume in ihrer Substanz hervorbringen, wie ich bei Arcella vulgaris und Okeni beobachtete. Tafel ix, fig. 1 ist eine A. vulgaris auf dem Rücken liegend abgebildet.' It appears from this statement that Perty knew of gas-vacuoles in Amoeba like those of Arcella.

Engelmann (1869, i and 1869, ii)[1] was the first to pay attention to the nature of the gas in the vacuoles of Arcella. He found that the gas-vacuoles disappeared when the Arcella containing them was killed either with caustic alkali or with acid, and states (1869, i, p. 311): 'Ueber die chemische Zusammensetzung der von den Arcellen entwickelten Luft habe ich ebensowenig als über den Mechanismus des Entstehens und Verschwindens der Gasblasen eine begründete Vermutung.'

Bütschli has described (1875) how he isolated a pair of deep brown Arcellae in conjugation which soon after separated. Each of these developed a large gas-bubble inside its test and one was seen to pour out its protoplasm in a broad stream from the opening of the test carrying with it the greater part of the large gas-bubble, although the bubble was certainly not enclosed in the protoplasm. In the evening the protoplasm returned to the test, carrying with it the gas-bubble. Bütschli found that the gas of the bubbles was gradually, steadily, and completely absorbed by caustic potash solution and considered this result conclusive evidence that the gas is CO_2, saying: 'Es kann unter den vorliegenden Umständen kaum zweifelhaft sein, dass wir es wohl mit Kohlensäure zu thun haben, dennoch muss es sehr auffallend erscheinen, dass diese Kohlensäureblasen so lange der Absorption durch das die Arcella umspülende Wasser widerstehen sollten' (Bütschli, 1875, p. 464). In his great work on Protozoa (vol. i, 1880–2, p. 101), Bütschli accepted the general view that the gas-bubbles existed as vacuoles in the protoplasm and makes the somewhat qualified statement: 'Ueber die Natur des entwickelten Gases liegen bis jetzt kaum Beobachtungen vor, Bütschli glaubt, wegen der raschen Absorption desselben durch Kalilauge, auf CO_2 schliessen zu dürfen.'

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[1] These two papers are identical, one being a translation of the other, but the French version (1869, ii) is illustrated by figures of Arcella containing gas-vacuoles (figs. 4 and 5, Pl. 36). These are wanting in the German paper (1869, i.).

Since Bütschli's observations were made only one other protozoologist, Khainsky (1910), has paid special attention to the subject. The following quotation gives the gist of Khainsky's conclusions regarding the origin and composition of the gas in the vacuoles of A. vulgaris (p. 170): 'Man möchte annehmen, dass die Gasblasen, welche gewöhnliche Luft enthalten, wie es auch Engelmann festgestellt hat, von aussen in die Arcellen eindringen. Eine solche Entstehung der Blasen habe ich experimentell bei Arcellen nachzuweisen vermocht. Wenn man aus dem Uhrschälchen, an dessen Boden die Arcellen kriechen, das Wasser absaugt und die Arcellenschale mit der äusseren Luft in Berührung tritt, so dringt diese durch die Schalenwand stürmisch hinein und bildet die genannten Gasblasen.' This passage shows clearly that what Khainsky dealt with in his experiments were not the gas-vacuoles in the protoplasm described by earlier observers, but air-bubbles, formed inside the test between it and the protoplasm, which displaced, as the air rushed in, the fluid which normally fills the space between the protoplasm and the test. Khainsky's reference to Engelmann's determination of the gas as air seems to be due to a misunderstanding by him of Engelmann's use of the word 'Luft'. This is obviously used in the old-fashioned wider chemical sense, meaning 'gas' and in his French version Engelman says: 'Relativement à la composition du gaz développé par les Arcelles, je n'ose hasarder aucune conjecture . . .' (1869, ii, p. 430).

I have repeated Khainsky's experiment of drying up Arcella and find that air enters the test under the conditions he describes, not, however, by 'violently penetrating the wall of the test', but by passing round the edge of the foramen. These artificial air-bubbles inside the test are, as Khainsky remarks, harmful and often fatal to the Arcella.

The only systematic experimental research on the gas-vacuoles of Arcella is that of Th. W. Engelmann (1869, i and 1869, ii). This research deals with A. vulgaris Ehrenberg as can be seen from the diagrams (1869, ii). It further can be concluded from the description (1869, ii, p. 424) that the whole of Engelmann's experiments were made on Arcellae which were artificially stimulated to form gas-vacuoles by a method probably discovered by chance, although Engelmann does not admit this. This is what I have called the method of reversal, i.e. turning over the Arcella so that it rests on the apex of the test, the pseudopodia which protrude through the foramen are directed upwards and find nothing solid to get a footing on.

A number of Arcellae were taken up in a pipette and put on the underside of a cover-glass in a hanging drop. The animals settled down on the surface-film of the drop, some of them foramen down and others reversed. The former crawled about on the surface-film of the drop while the latter showed the phenomena described in the rest of the paper.

The reversed Arcella 'after a certain time (from two to about fifteen minutes) during which the animal remains motionless or makes vain efforts to reach a fixed point with its pseudopodia, a certain number (usually two to five, sometimes up to fourteen) of dark spots appear simultaneously or at short intervals of time in the protoplasm. These spots are almost always situated at a short distance from the periphery of the protoplasm contained in the test, and often at very regular distances one from the other. A few minutes afterwards one observes that the dark spots enlarge and swell into very distinct gas-bubbles with black outlines and generally irregularly spheroidal (see 1869, ii, figs. 4 and 5, Pl. 36). Sometimes the volume of these gas-bubbles increases so much that they occupy a considerable portion of the space inside the shell, so that a part of the protoplasm usually contained within the test is, of course, pushed out of it. When the gas-bubbles are few in number they all reach a considerable size (about 0·06 mm.). When they are numerous the individual bubbles remain smaller (0·01 to 0·02 mm. approximately). They are always surrounded on all sides by protoplasm. When the bubbles have reached about their maximum size, generally five to twenty minutes after their appearance, the Arcella begins to float up slowly and often with gradually increasing speed, rising vertically in the drop until it reaches the upper surface. When the opening of the test is turned upwards, the Arcella succeeds in attaching itself by its pseudopodia and creeps away. When this happens the gas-bubbles soon become smaller, in five to ten minutes they have, as a rule, become reduced to a diameter of scarcely 0·002 mm. and they now disappear with a sudden rush. If the Arcella, however, finishes its ascent with the dorsal side uppermost, the volume of the gas-bubbles does not diminish so long as the animal cannot turn over and fix itself to the surface above it. Once the Arcella has lost its gas and is fixed, if one detaches it by shaking the drop or by touching the animal gently with the point of a fine needle, it immediately falls to the bottom of the drop. After an interval, often of a few minutes only, gas-bubbles form anew in the protoplasm which soon again float the Arcella up to the surface above.'

My own observations on A. discoides in a hanging drop agree, as regards behaviour, exactly with Engelmann's so far as described above. His next observation on A. vulgaris I have not been able to confirm in A. discoides. He says that

'When the Arcella is lying on its back at the bottom of the drop, it sometimes happens that one bubble or a small number of bubbles form only on one side. In a short time the animal begins to rise at this side and so stands on its sharp edge. In this position it usually succeeds in reaching with its processes a fixed point on the lower surface of the drop and this allows it to finish righting itself so that the central side is turned down. Immediately the gas-bubbles become smaller and generally disappear in the next few minutes.'

In the large number of observations made on A. discoides

I never observed that the position of the gas-bubble, when only one was present, had any influence on the point of the circumference which became uppermost as the animal righted itself; but without observations on A. vulgaris I would not like to dispute the accuracy of Engelmann's observations on this species. As a matter of fact, A. discoides in my experiments never succeeded in righting itself when lying on the surface-film of the hanging drop, foramen uppermost, when the surface of the drop was perfectly clean. The Rhizopod only succeeded in righting itself when one of its pseudopodia, bending over the edge of the test and stretching down to the surface-film, happened to attach itself to some solid body such as a diatom held there by surface tension.

According to Engelmann:

> 'An analogous phenomenon is seen in some of the Arcellae which have been floated up to the top of the drop, dorsal side uppermost. After they have exhausted themselves for some time in useless efforts to attach themselves by their pseudopodia, the gas-bubbles diminish in volume on one side. Consequently, this side sinks, the Arcella takes up an inclined position, then a vertical one and finally turns over completely as soon as one of its pseudopodia has fixed itself. Then the gas-bubbles disappear rapidly.'

From the records of hundreds of righting experiments made on A. discoides, I am forced to the conclusion that the observations just described result from a purely fortuitous arrangement of the gas-vacuoles in Engelmann's A. vulgaris.

Engelmann continues:

> 'The bubbles of gas generally only arise and expand when the Arcella cannot attach itself and move about by means of its pseudopodia. The gas-bubbles diminish in size as soon as the pseudopodia have found a fixed point. By taking this circumstance into account, it is possible to predict with almost absolute certainty whether an Arcella will form gas or not and should gas-bubbles be present already, to predict whether these will enlarge or shrink. By putting the animals artificially into different positions (for instance, by means of a needle under a simple microscope) one can, at will, cause the bubbles to appear or disappear, to become larger or smaller.'

Engelmann's careful observations will be referred to as occasion arises in the course of this paper and his accuracy duly acknowledged. It may, however, be pointed out here that he leaves no doubt about the fact that the gas-bubbles are formed in the substance of the protoplasm, that they remain imbedded in the protoplasm and are there absorbed. In his fig. 5, Pl. 36 (1869, ii) he gives a radial optical section through an Arcella passing through two gas-vacuoles. The figure is from a somewhat rough sketch, but is quite correct so far as it goes. The smaller of the two gas-bubbles is distinctly shown as completely enclosed within the protoplasm.

Perty, Engelmann, and Bütschli agree in attributing a hydrostatic function to the gas-vacuoles. Engelmann, indeed, goes so far as to say: 'Man kann nicht läugnen, dass diese Thatsachen auf psychische Processe im Protoplasma deuten' (1869, i, p. 311); the facts alluded to conveying a strong semblance of purposeful action in the formation of the gas-vacuoles when and where they are required to assist in attaching a pseudopodium and in their disappearance, without any other apparent reason, as soon as they are no longer needed. Engelmann describes also how he found Arcella containing gas-vacuoles at the surface of the piece of water in which they live, showing that they not only produce and absorb gas under experimental conditions but also use this method of altering their specific gravity in their natural surroundings.

CHAPTER II.—MATERIAL.

All my observations have been made on A. discoides Ehrenberg, a species which is in some respect superior to A. vulgaris for observation and experiment. The test of A. discoides as compared with the test of A. vulgaris is more transparent, much paler in colour, and flatter; a daughter A. discoides newly formed by fission has a perfectly colourless test or one only very faintly tinged with yellow. I succeeded in keeping up a constant supply of these daughter Arcellae by a method described below. Another advantage which A. discoides has over A. vulgaris consists in the shape of the protoplasm inside the test, this being spread out flatter and thinner instead of being curved and dome-shaped as it usually is in A. vulgaris. This flat shape obviously facilitates observations on the living animal under high powers of the microscope.

The original stocks all came from running water. They were found on the leaves of Fontinalis antipyretica growing in the Thames near Iffley, on Elodea canadensis from a tributary of the Cam running through Shepreth, and on Fontinalis from the Granta close to Cambridge. Cultures from these sources were kept in small aquaria (9-inch bell-jars) filled with rain-water. The plants were given no earth as the water might have dissolved out substances from it which could discolour the tests of the Arcellae.

A simple method of obtaining a continuous supply of suitable specimens was adopted. A number of watch-glass cultures were kept in a moist chamber consisting of a 10-inch Koch culture-dish with damp filter paper spread on the inside of the cover. These small cultures were started by putting into a corked tube as much filtered rain-water as a watch-glass would hold with a little of the weed from an aquarium, the size of the tube being such that it was now half full. A few vigorous shakes of the tube detached not only all the Arcellae, but also quantities of the diatoms, desmids, and Chlorophyceae on which Arcella feeds ; the turbid water was poured into a watch-glass at once. Using the watch-glasses in rotation and adding filtered rain-water as required, these cultures went on yielding daughter Arcellae for weeks. It was found to be necessary to remove from the watch-glasses any small crustaceans and molluscs which would otherwise soon have cleared away all the Algae and left the Arcellae to starve. Then the Algae would often multiply faster than the Arcellae could consume them. All the cultures were kept near windows with a northerly aspect.

CHAPTER III.—MORPHOLOGY OF A. DISCOIDES EHRENBERG.

When describing the morphology or functions of Arcella or any similar Rhizopod it is necessary to use terms to distinguish the two sides usually called 'dorsal' and 'ventral'. There are many objections to the use of these terms as applied to Protozoa. After rejecting them (and others), I have found, by the test of experience, that the words 'obverse' for 'dorsal', and 'reverse' for 'ventral', are suitable and expressive. The obverse is the domed roof of the Arcella test which is normally turned towards the outer world, while the reverse is turned towards the surface upon which the Arcella crawls (fig. 4, Pl. 31).

The diameter of the test measured in a few specimens was 0·1 millimetre. In shape the test is more depressed than that of A. vulgaris and the reverse of the test is not flat as in that species but is turned into the test forming a shallow inverted funnel (fig. 4, Pl. 31). The opening of the test, the foramen, is thus tucked up into the test-cavity and the funnel depression accommodates the locomotor (external) pseudopodia and allows them to act without lifting the outer edge of the test much above the surface upon which the Arcella is crawling (fig. 1 F, Pl. 30). The 'engine-turned' markings on the test are in this species very delicate (fig. 3, Pl. 31).

As a rule the protoplasm does not fill the whole of the test, but leaves a space, the test-cavity, which is filled with a watery fluid. In A. discoides a cast of this space would be roughly annular and it is referred to here as 'the marginal space' (fig. 4, Pl. 31). The way in which the space within the test is cramped by the funnel projecting into it may be one reason, the flat dome may be another, why there is, in this species, no cavity between the obverse surface of the protoplasm and the apical region of the test. The cylinder of protoplasm which completely fills the test over the area of the foramen will be called 'the central mass' (fig. 4, Pl. 31). The ring of protoplasm round the central mass will be called 'the outer zone'. Its obverse surface is not in contact with the test, except where pseudopodia are formed from it to reach across the marginal space. These pseudopodia I call ‘ internal pseudopodia ’ (fig. 4, Pl. 31) to distinguish them from the locomotor pseudopodia which are extruded through the foramen and may be called ‘ external pseudopodia ’. Some of the internal pseudopodia may persist for hours when the tip touches the test. These persistent ones mostly arise from the extreme edge of the outer zone (fig. 3, *m.p.*^{10}, Pl. 31). As this edge is of special physiological importance, it requires a name and it will be referred to as ‘ the marginal zone ’ (fig. 4, Pl. 31). Its width can be defined as equal to the diameter of the contractile vacuoles at diastole (fig. 3, *c.v.*^{9}, Pl. 31). The majority of these vacuoles, there may be as many as fifteen or sixteen present, lie against the extreme edge of the outer zone. Very occasionally, however, one or two have been found discharging their contents on the reverse surface of the outer zone, indicating that the protoplasm inside the marginal zone is not always in contact with the funnel. These contractile vacuoles were close to the marginal zone. Put briefly, the protoplasm is in contact with the test in two areas, in the apical area and in the zone of the funnel round the foramen (fig. 4, Pl. 31).

All the relations described above are of functional significance; it is therefore necessary to point out that any figure showing an A. discoides in transverse section with a space between the obverse surface of the central mass and the test is not correct. This applies to all the published figures I have seen. The refractive qualities of the test make it impossible to see the protoplasm distinctly in a median optical section of A. discoides viewed edgeways. The only way to observe accurately the relations of protoplasm and test is to use a high power with a slow fine adjustment on living and on well-preserved specimens as they lie flat, and the above conclusions are the result of a large number of such observations.

The number of nuclei was invariably two, except in cases of incomplete fission, where there are four. The nuclei always lie in the outer zone of protoplasm and generally, but not always, on opposite halves of a diameter. In fig. 3, Pl. 31, they do not lie on a diameter. As the protoplasm is fluid, the position of the nuclei, like that of all other inclusions, is constantly changing, yet there are no active streaming movements of the endoplasm. Each nucleus contains a large karyosome with spoke-like processes radiating out to the surface of the nucleus, as seen in many groups of the Protozoa (fig. 3, Pl. 31).

The descriptions of the gas-vacuoles, pseudopodial movements, behaviour of the contractile vacuoles, and so on will follow in the account of the results of experiments.

A few other new terms which it has been found necessary to introduce will be best explained as they come into use in the course of the descriptions or argument.

When noting the position of any feature of the organism it was found to be useful to imagine the numbers 1 to 12 arranged round the edge of the test as on a clock dial and to number any particular feature accordingly. In fig. 3, Pl. 31, the three external pseudopodia would be called *e.p.*^{11}, *e.p.*^{3}, and *e.p.*^{7}, from the position of their tips.

CHAPTER IV.—THE FORMATION AND ABSORPTION OF GAS-VACUOLES

A. The Standard Reversal Experiment.

(*a*) Description of the Standard Experiment.—A uniform method of stimulating Arcella to form gas-vacuoles was adopted. Throughout the experiments now to be described it was closely adhered to, for three reasons. In the first place it proved to be most convenient. Secondly, by keeping the method of stimulation as nearly as possible uniform, the reactions under the same and under varying conditions could be more legitimately compared, having first established a standard under uniform conditions. Thirdly, the method of stimulation being essentially a mechanical one, does not admit of any small changes of concentration or intensity liable to crop up if a chemical or physical stimulating agent were used. The method was one of Engelmann's (p. 309, 1869, ii) slightly modified. Engelmann's experiments were nearly all performed on A. vulgaris in hanging drops. I have used the hanging-drop method for special purposes, but in all other cases, unless the contrary is expressly stated, the following course was adopted. I shall refer to it as 'standard method'.

Three or four drops of carefully filtered rain-water were put on a slide, a selected Arcella from the bottom of a watch-glass culture under a Greenhough binocular was taken up in a fine pipette (bore about 0·5 mm.), with as little water as possible and put into the rain-water on the slide. The water from the culture was not used as it is apt to contain diatoms and other Algae, which are not wanted on the slide with the Arcella. The specimen was then examined under the microscope to determine whether it was lying foramen up. If not, the Arcella was reversed by gently pushing it with a fine needle under a low power (ap. 16 mm., comp. oc. 2). It was then watched under a higher power (ap. 8 mm., comp. oc. 12). No cover-glass was used and evaporation was checked by the occasional addition of a drop of filtered rain-water. The time of reversal was noted in each experiment, also the times of the various reactions in the protoplasm which take place in orderly sequence during the process of righting itself which every Arcella carries out.

Looking down on the reverse surface of the Arcella with the microscope and constantly focussing up and down is the best method for following all the movements of the protoplasm, but for observing the actual method of righting itself used by the Arcella it is best to watch the animal edgeways. This can be done under a low power by reversing with a needle an Arcella on the bottom of a rectangular trough clamped to the vertical stage of a horizontal microscope.

The normal flat A. discoides rests, when reversed, on the apex of its test in a perfectly horizontal position. Very soon, usually one or two minutes after reversal, the external pseudopodia are protruded (fig. 1 A, Pl. 30). They are always withdrawn during the reversing process. They are vigorously waved about in the water as though they were groping for a solid object above the animal. Others stream out towards the edge of the test and beyond it, and one of these, bending downwards, comes into contact with the glass, attaches itself and depresses the edge of the test over which it passes (fig. 1 A, Pl. 30) as it becomes shorter and thicker. By this time, from five to twenty minutes after reversal, gas-vacuoles may or may not have formed, but in any case the whole animal is raised bodily from the bottom (fig. 1 B, Pl. 30) by the attached pseudopodium and swung over first into the vertical position (fig. 1 C, Pl. 30) and then beyond this so that the animal rapidly falls with its foramen down (figs. 1 D and 1 F, Pl. 30). The external pseudopodia are then partly withdrawn (fig. 1 F, Pl. 30), the animal settles down on the bottom and crawls away in the manner characteristic of A. discoides, that is with the pseudopodia only rarely showing their tips beyond the edge of the test. In fig. 1 C, D, and E, Pl. 30, it will be seen that the attached pseudopodium has rapidly thickened towards the obverse surface and has even branched out in this direction. The other external pseudopodia are moving actively during these stages. The whole of the evolution of righting shown in figs. 1 A to E, Pl. 30, takes place in one and a half to two minutes. The considerable extension of the pseudopodia and their vigorous, unceasing movements before and during the righting process produce an impression of great activity and expenditure of energy on the part of the Arcella.

Watching the reversed Arcella on a slide the protrusion of active pseudopodia from the foramen is seen to be accompanied by changes in the protoplasm left within the test. The bulk of this is, of course, reduced by the amount protruded through the foramen and consequently the marginal space becomes larger: this marginal widening is generally quite obvious, it makes room for the ensuing expansion of the internal protoplasm when the gas-vacuoles swell out its bulk at the expense of the fluid in the marginal space. As the external pseudopodia are always formed before the gas-vacuoles appear the space for expansion is provided in advance. The first indication of the formation of gas-vacuoles in a suitable specimen of Arcella such as is shown in fig. 3, Pl. 31, is the rapid protrusion of marginal lobopodia into the marginal space at the points of the circumference where the gas-vacuoles are about to form. The appearance of these marginal lobopodia is quite different from that of the ordinary marginal pseudopodia (fig. 3, *m.p.*^{2} and *m.p.*^{7}, Pl. 31) which are more or less persistent filopodia with a conical base. *m.p.*^{4}, *m.p.*^{5}, and *m.p.*^{10} of the same figure are hyaline marginal lobopodia bearing radiating filopodia. At the base of the lobopod the granular portion of the protoplasm is sharply demarcated. The lobopodia execute rapid amoeboid movements, especially rapid in throwing out filopodia and thickening them (*m.p.*^{9}, *m.p.*^{10}) or thinning them down and withdrawing them, to throw out others at different points on the surface of the lobopod, at the same time the lobopod itself constantly changes its shape. It is at the base of such marginal lobopodia that the gas-vacuoles may be expected to appear. Where two such pseudopodia are confluent as at *m.p.*^{9} and *m.p.*^{10} (fig. 3, Pl. 31), the chances are that two gas-vacuoles will arise close together. Sometimes a marginal pseudopodium is put out and no gas-vacuole is formed, but in such specimens as this (fig. 3, Pl. 31) each gas-vacuole is heralded, as it were, by an active marginal lobopodium.

In fig. 2, Pl. 30, a series of rapid but careful sketches are shown of the movements of the protoplasm before, during, and after the existence of a small, short-lived gas-vacuole. Fig. 2 A, Pl. 30, was drawn just before the gas-vacuole shown in fig. 2 B, Pl. 30, became visible, so that the position of the minute vacuole could be accurately marked in the same outline sketch opposite the middle of the base of the largest marginal lobopod (fig. 2 B, *g.v.*, Pl. 30). The minute gas-bubble appears as a black spot which can readily be distinguished by its rapid increase in size from the granules in the protoplasm, which also look black by transmitted light. About thirty seconds after its appearance the gas-vacuole had grown to such a size that light was transmitted through its central portion. The edge of the gas-vacuole always remains dark, like the edge of all gas-bubbles under the microscope (figs. 2 C, D, and E, Pl. 30). While the gas-vacuole was expanding the marginal protoplasm near it was quite turbulent in its movements (figs. 2 B, C, and D, Pl. 30). The shapes of the lobopodia were changed from second to second and the filopodia were extended and retracted with such rapidity that only a cinematographic series of photographs could adequately represent their movements. Figs. 2 B, C, and D, Pl. 30, give some slight indication of the type of pseudopodium formed. To give an idea of the speed of the movements, it may help by stating that the notch opposite the gas-vacuole in fig. 2 D, Pl. 30, formed and was obliterated in about ten seconds. In this fig. 2 D, Pl. 30, the gas-vacuole is shown at its largest size; it is not so large as the contractile vacuole lying next to it (see p. 539). The shell of clear protoplasm without granules which envelops the gas-vacuole is represented in figs. 2 C, D, and E, Pl. 30. In fig. 2 D, Pl. 30, an indication can be seen of streaks radiating out from the surface of the clear protoplasm enveloping the vacuole. When the gas-vacuole began to dwindle these streaks went. The gas-vacuole disappeared about three minutes after reaching its full size (10 min. 30 sec. after reversal), leaving a pear-shaped clear patch of protoplasm in the space it had occupied (fig. 2 F, Pl. 30). If this figure (F) is compared with the two preceding figures (E and D) it will be seen that the marginal protoplasm is still tending to extend outwards during the time the gas-vacuole is shrinking and disappearing. This extension of the protoplasm is shown (1) by the broadening of the lobopod seen by comparing fig. 2 D with figs. 2 E and F, Pl. 30; the contractile vacuole in D is in F carried out into the lobopod towards the edge of the test; (2) by the thickening of the filopodia (see figs. 2 E and F). These are both signs that the main flow of the protoplasm in this part of the marginal zone is still centrifugal. The clear protoplasm left where the gas-vacuole had been became granular and indistinguishable from the surrounding protoplasm one and a half minutes after the gas-vacuole had vanished (fig. 2 G, Pl. 30). By this time also the broad lobopod had been withdrawn and was now a small protuberance bearing about a dozen attenuated filopodia, some of them reached the test, others had delicate free tips and these were rapid in their movements. The sketch (fig. 2 H, Pl. 30) made one and a half minutes later shows most of the filopodia withdrawn, others almost retracted, while two or three still reached the test; one or two of these may remain as persistent marginal pseudopodia, but in another one or two minutes the short filopodia had all gone, and five minutes after the time when the gas-vacuole vanished the marginal protoplasm had resumed its normal appearance without any sign of a gas-vacuole having been there. The Arcella continued to lie with its foramen up for another four minutes or thereabouts, and then righted itself nineteen minutes after reversal without having formed another gas-vacuole before the tilting movements began. However, three minutes before this, i. e. sixteen minutes after reversal, movements of the marginal protoplasm similar to those indicated in fig. 2 A, Pl. 30, were observed, but no gas-vacuole was visible until the Arcella had turned itself over, when a minute one was seen to have developed (while the righting movements were going on) at the part of the marginal zone where the marginal pseudopodia had last been observed.

This retarded development of gas-vacuoles will be discussed later in connexion with the nature of the conditions which lead to the evolution of gas in the protoplasm.

In the experiment just described the gas-vacuole played little or no part in the righting process, but a great majority of the experiments showed that as a rule the gas-vacuoles are concerned in the righting process. One of these experiments will now be described with the help of the series of diagrams (figs. 5 A–P, Pl. 32). The diagrams are reproductions of rapid sketches made from an Arcella which, when turned foramen up, formed five large gas-vacuoles, began to turn itself over when the gas-vacuoles were reaching their maximum size, and rapidly absorbed them when it had completely righted itself.

This Arcella, when turned over with a needle, put out its external pseudopodia in the usual way, but special attention was given only to the marginal zone of the internal protoplasm. The edge of the internal protoplasm was at the time of reversal roughly concentric with or parallel to the edge of the test (as shown in fig. 5 P, Pl. 32), but about three minutes after reversal a large protuberance was formed extending over nearly one-third of the circumference (fig. 5 A, Pl. 32). This broad and short lobopod bore on its edge small blunt lobopodia especially well developed on its left-hand edge; these small lobopodia each again threw out a tuft of fine filopodia which came into contact distally with the internal surface of the test. Within a minute after this condition had been sketched (in fig. 5 A, Pl. 32) a gas-vacuole appeared in the protoplasm at the centre of the left-hand region of the large protuberance, where, judging by the movements of the pseudopodia, the protoplasm was most active (fig. 5 B, Pl. 32). About one and a half minutes after the appearance of the first gas-vacuole a second one formed in the right-hand half of the large protuberance (fig. 5 C, Pl. 32) in the protoplasm near the bases of a rather less well-marked group of lobopodia than those on the left-hand half. Meanwhile the first-formed gas-vacuole had enlarged and for another four and a half minutes (figs. 5 D and E, Pl. 32) both of them continued to expand, at the same time each found room for its expansion in a marginal lobopodium (figs. 5 D and E, Pl. 32). The latter sketch shows how close to the surface the gas-vacuoles lie, because they happen to have remained at the horizontal level of the edge of the protoplasm as they enlarged. During the next two minutes the first-formed gas-vacuole quickly diminished to less than half its former diameter (fig. 5 F, Pl. 32). The next sketch (fig. 5 G, Pl. 32), made one and a half minutes later, shows that both gas-vacuoles were now dwindling, but a comparison of the two preceding sketches (E and F) shows that one of the gas-vacuoles had begun to contract while the other was still expanding and that the latter was the one to the right which was formed later than the other. Fig. 5 H, Pl. 32, shows that shrinkage continued for another two minutes but more slowly than before, and then both gas-vacuoles began to dilate again (fig. 5 I, Pl. 32). This phase continued for about five minutes before it was completed; however, the Arcella succeeded in righting itself nineteen minutes after it had been reversed. The two gas-vacuoles under observation continued their expansion after the Arcella had turned itself foramen down until twenty and a half minutes after reversal (fig. 5 J, Pl. 32). They did not enlarge beyond the size represented in this sketch, but persisted for about a minute and then commenced to diminish (see figs. 5 K and 5 M, Pl. 32) steadily and without interruption until they vanished almost simultaneously (fig. 5 N, Pl. 32) leaving a small clear patch of protoplasm marking the place where each disappeared.

The existence of these two gas-vacuoles constituted an episode of very rare occurrence in the observations made on some hundreds of gas-vacuoles in Arcella under the conditions of the standard experiment. The interruption of the expansion in both gas-vacuoles by a short period of shrinking corresponds to a process obtained under abnormal experimental conditions to be described later in connexion with the behaviour of the gas-vacuoles in the presence of vital stains.

The usual course of events under the normal conditions of the standard experiment can be seen in fig. 5, Pl. 32, if the figures lettered F, G, and H are disregarded and the stages followed from figs. 5 A to E, and then from figs. 5 I to P.

The movements of the marginal pseudopodia before the appearance of the gas-vacuoles have already been described. These movements in the immediate neighbourhood of the gas-vacuoles continue and are extremely active during both the expansion and contraction periods. There appears to be no cessation of activity during either of these periods, nor is there any marked increase or decrease at any particular phase of the gas-vacuoles. In some specimens there is possibly a slight increase of activity in the production and motility of filopodia at the time when the gas-vacuoles are beginning to contract. An Arcella containing gas-vacuoles in this phase was preserved and is figured (fig. 6, Pl. 33). Just before this young daughter Arcella was killed the marginal protoplasm was in very active movement in the immediate neighbourhood of each of the four vacuoles. In the stained and mounted specimen the condition of the external and internal pseudopodia is preserved as in life. Each of the four gas-vacuoles extends into a lobopod bearing on its surface so many secondary pseudopodia that the lobopodia have quite a tufted appearance. The secondary pseudopodia are for the most part delicate filopodia arising from a conical base. There is a marked contrast between the appearance of the surface of the vacuolated lobopods and the surface of the marginal protoplasm extending between them, where the smooth outline indicates quiescence. During life the distinction is, of course, much more marked in consequence of the lively movements of the filopodia.

As in the case just described, so in most of the others which came under observation, the gas-vacuoles were at their largest at the time the Arcella righted itself. There can be no doubt that the presence of gas-spaces in the protoplasm during the time it is turning itself over, assists the Arcella to accomplish this act with a reduced expenditure of energy, as the volume of gas is sufficient to lower the specific gravity of the Arcella to the specific gravity of water or below it according to the quantity of gas evolved. Thus every Arcella in these experiments which rights itself while the gas-vacuoles are large has the advantage of being floated up until it stands on edge and then the weight of the pseudopodia extended out from the foramen (as shown in fig. 1, Pl. 30) brings down the Arcella into its normal position. I have never observed a failure to come down obverse side up in consequence of the shifting of the centre of gravity into the base of the external pseudopodia, when once the Arcella had risen to stand on edge. But in half a dozen instances the Arcella has lost its hold on the glass, perhaps on account of its great buoyancy, and floated up to the surface of the drop.

Arcella, when reversed in the standard experiment, always rights itself and generally, but not always, forms gas-vacuoles before beginning to turn itself over. Sometimes these vacuoles do not appear until the Arcella has started righting itself, and they may, in exceptional cases, appear after the Arcella has turned right over and is crawling away. A few such belated appearances are included in the table (Table I) given below. This table condenses the results of 101 reversals practised, under the conditions of the standard experiment, on 74 individuals and shows how often a negative or a positive result, as regards gas-vacuole production, was obtained. When the same Arcella was reversed more than once the results of a reversal were allowed to die away completely before the next reversal was carried out; if gas-vacuoles had formed they were followed until completely absorbed, and in all cases the Arcella was not turned over again until it was normally crawling along.

The total at the bottom of Table I show how the individual Arcellae behaved when righting themselves.

Table I.

Production of gas-vacuoles by Arcella in the standard reversal experiment.

Numbers of consecutive reversals.	*Result of experiment.*	*Reversed once.*	*Reversed twice.*	*Reversed thrice.*	*Reversed four times.*	*Total results.* +	*Total results.* −	*Total number of reversals.*
First	+	48	5	2		55		74 first
	−	7	3 6		2 1		19	
Second	+		5 6	2	1	14		19 second
	−		3		2		5	
Third	+			2		2		5 third
	−				2 1		3	
Fourth	+				2 1	3		3 fourth
	−							
						74	27	101

Reversal formulae of individual Arcellae	48 + 7 −	5 + + 6 − + 3 − −	2 + + +	2 − − − + 1 − + − +	55 + results only 9 − and + results 10 − results only
Totals	55	14	2	3	74 individuals

The + and — signs signify a positive or negative result respectively as regards the formation of gas-vacuoles.

The upper part of the table shows the result of each reversal, but those individuals which behaved alike are grouped together as can be seen from the lower part of the table where the performance of the individuals is given.

The results may be stated as follows:

(1) Out of 101 reversals, 74 resulted in gas-vacuole formation, 27 did not.

(2) The 74 individuals experimented on included 10 which failed to form gas-vacuoles, 7 of these were only reversed once, 3 only twice.

(3) Of the remaining 64 individuals, 55 always gave positive results, the remaining 9 were (*a*) 7 which gave a positive result only on the second reversal, and (*b*) 2 which did not form gas-vacuoles until the fourth reversal.

It is clearly not safe to conclude from the above that some Arcellae are unable to produce gas-vacuoles. In 48 other individuals than the above treated with vital stains, &c., but otherwise reversed under the conditions of the standard experiment, only one Arcella reversed twice failed to produce gas-vacuoles; altogether then, out of a total of 122 Arcellae there were only 10 which failed to react in this way. These 10 might, like the 2 Arcellae which produced gas-vacuoles on the fourth reversal, also have produced them if they had been reversed more than once or twice. The question naturally arises: Are the gas-vacuoles to be considered as adaptive organs, used, not only to float Arcella up to the surface of the water, but also to facilitate or expedite the righting process when it has by accident been reversed? The bearings of this question are important. It seems most improbable that Arcella should ever have any difficulty in righting itself in a few seconds should it ever be accidentally turned over in its natural surroundings, i.e. the felt or ooze of Algae where it feeds, or on the surface-film, when it has floated itself up and found itself at the surface wrong way up. In such cases the gas-vacuoles are already present and, moreover, the surface-film is never quite clean as it should be in Engelmann's hanging-drop experiment. As I have observed when diatoms, Algae, or any solid particles are on the surface-film the Arcella lying reverse side up, can get a hold with its pseudopodia and turn over quickly without forming gas-vacuoles. That is to say, the gas-vacuoles make their appearance only when the Arcella fails to right itself quickly, or putting the case differently, it forms the vacuoles if, for a sufficient length of time, the external pseudopodia are not in contact with a solid substratum. That the substratum must be one to which the pseudopodia can adhere is shown by Engelmann's experiment, and, so far as is known, this condition is only fulfilled when the substratum is a solid one. The pseudopodia appear to be unable to attach themselves to the clear surface-film of the hanging drop. This is of theoretical importance as will be shown later.

Engelmann's observations on the influence of the position of the gas-vacuole when a single one is present in the act of tilting, i. e. raising the edge where the gas-vacuole lies, I have found to be, on the whole, not applicable in A. discoides, as the tilting and righting depends chiefly on the activity of the pseudopodia. It appears from a large number of records of my experiments to be usual that the side of the reversed Arcella which is depressed is that on which the pseudopodia are most active, and it may sometimes happen that the opposite side contains the largest gas-vacuole. Should this be the case, however, it is probably a mere coincidence, as in a very large number of cases the largest gas-vacuole is on the same side as the most active pseudopodium. It should be noted here that attention has been paid to the question whether the most active pseudopodium in the reversed Arcella is the one which anchors itself and is instrumental in righting the Arcella, and this is almost always the case.

The circumstances just described show that the gas-vacuoles are formed, not as an adaptation to be used in facilitating the righting of a reversed Arcella, but as a by-product resulting from the main activity of the organism for the time being, which consists in the struggle to turn over into the crawling position. If the other organisms which form gas-vacuoles, Difflugia, Amoeba, &c., are considered, it is obvious that, as in their case, there can be no question of reversing and righting, the formation of gas-vacuoles in all these Rhizopods is due to the same set of causes.

The further discussion of these causes must be deferred until the end of the paper.

(*b*) The Reaction Time and Latent Period of the Gas-vacuole.—In dealing with these time relations, stimulation is considered to be applied at the moment when reversal takes place. This is clearly the only precise time which can be fixed, and the response in the formation of the gas-vacuole follows occasionally so quickly that there is practically no choice in this matter. As gas-vacuoles may form within one minute after reversal, there is clearly no time to note anything which may take place during this minute, the greater part of which is taken up with the necessary manipulations of the microscope. The results of 110 cases in which the time of gas-vacuole formation was carefully recorded show that the time of appearance of the first vacuole varied between the first minute and the fourteenth from zero time. The grouping of the times between these limits is shown in Table II. In the lower part of this table the minutes are placed together in groups of three, as this brings out more clearly the most frequent interval between stimulation and response. This is shown to lie between the beginning of the fourth and the end of the sixth minutes. The response, therefore, is extremely slow as a physiological response. However, the approximate stimulus takes place later than has been assumed. It commences, as will be shown in the general discussion, when the pseudopodial activity commences. The latent period of gas-vacuole formation, therefore, is shorter than the figures in Table II indicate. A reduction of the latent period, as shown in these figures, must

TABLE II.

To show (I) how often the first gas-vacuole appeared in each minute after reversal, and below (II) how often in each group of three minutes; the results are of 110 reversal experiments.

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14 Minutes
I	3	4	7	16	17	20	11	9	6	9	2	4	1	1	= 110 total
II	14			53			26			15			2		

also be made at the latter end of this period. The first appearance of the gas-vacuole as a minute black dot among other equally dark granules in the protoplasm is very difficult to determine accurately, as the only distinction which can be observed at this early stage is the fact that the minute gas-vacuole increases in size while the granules near it remain stationary in size, but not always in position. The shifting of these granules sometimes, when their shape is irregular, involves an appearance of growth, for instance, when such a granule rotates, presenting first a smaller side and then a larger one. Another difficulty in distinguishing between the minute gas-vacuole and a granule is due to the frequent non-expanding, stationary condition of the gas-vacuole itself at this early stage. These circumstances tend to delay the recognition of the gas-vacuole, and this tends to lengthen the apparent latent period. All the figures in Table II are therefore only approximations and are weighted on the same side. In some of the experiments the gas-vacuoles remained in the stage of a black dot for as long as five minutes, and were only recognized, not having expanded, by the facts that marginal pseudopodia were formed and disappeared in time with the appearance and disappearance of the gas-vacuole associated with it. This shows that the figures on the right-hand side of the table may be, and probably are, too numerous, making the curve of frequency more symmetrical than it should be. The mode of the curve would therefore be shifted to the left, and increase the number of the mean time of appearance.

(*c*) The Influence of Gas-vacuoles on the Time of Righting.—As 122 experiments were available in which the time taken by the reversed Arcella to right itself was recorded, some conclusion may possibly be drawn as to whether the presence of gas-vacuoles tends to shorten the reversed period. Taking all the experiments together, the average time in the reversed position where no gas-vacuoles were formed before righting was 12·24 minutes. This applies to 39 experiments. The average time in the same position of those with gas-vacuoles was 10·08 minutes, which indicates that the gas-vacuole formation goes with speedy righting. These 122 experiments, however, were not all standard experiments, and it would perhaps be advisable to omit all experiments under abnormal conditions. This leaves 82 standard experiments in which the time average, taking those which had no vacuoles as 100, was in the ratio of 100 to 72·5. The ratio of the whole 122 experiments, expressed in the same way, is 100 to 82·35. The abnormal conditions referred to above were such as to limit the activity of the protoplasm, and it is therefore not surprising that in the treated specimens the righting process should be delayed.

(*d*) Synchrony of Gas-vacuole Formation.—The great majority of the experiments in which gas-vacuoles appeared showed very clearly that whatever the determining cause for their formation may be, it is one which does not act locally in the protoplasm, but on the organism as a whole. This can be deduced from the very numerous instances where the gas-vacuoles either appeared simultaneously or in rapid succession within a short interval of time.

(*e*) Constant Features of the Standard Reversal Experiment.—In all these experiments carried out under conditions which were practically uniform, the results were also uniform to an extent which is indicated in Table I. In the larger group containing those Arcellae which did form gas-vacuoles, some of the results of reversal depended on the amount of protoplasm contained within the test, more than on any other factor. These individuals, when the test was completely filled with protoplasm occluding all the marginal space, could not, of course, form marginal pseudopodia. In other respects the features enumerated below were shown in the same way in these fully grown Arcellae, as in the young daughter Arcellae with the tests only half filled with protoplasm. A summary of the constantly recurring series of events after reversal in such daughter Arcellae is given under the following twelve heads:

(1) External pseudopodia are protruded before the formation of the gas-vacuoles.

(2) Marginal pseudopodia are protruded later than the

external pseudopodia but before the appearance of the gas-vacuoles.

(3) The position of the gas-vacuoles at the time of their appearance is constant, viz. always in the marginal zone.

(4) The position of the vacuoles at the time of their disappearance is constant, viz. always in the marginal zone.

(5) The position of the vacuoles when fully formed is always in the outer zone, never in the central mass.

(6) The gas-vacuoles grow steadily until reaching their maximum size.

(7) The gas-vacuoles diminish steadily until their final disappearance.

(8) When several gas-vacuoles are present their appearance, growth, dwindling, and disappearance roughly synchronize.

(9) The gas-vacuoles do not persist after the Arcella has resumed its normal crawling state.

(10) The activity of the marginal pseudopodia during the presence of the gas-vacuoles whether they are growing or shrinking is constant.

(11) The retraction of the marginal pseudopodia after the disappearance of the gas-vacuoles is invariable.

(12) The regular appearance of a notch or crater in the marginal pseudopodium at an early stage of the expanding gas-vacuole.

All the above activities are carried out in these experiments as though the Arcella were following a regular programme. In only two of these phases of activity did any variation take place; under heads 6 and 7 two cases of exception were found in two experiments. One of these exceptional cases is illustrated in fig. 5, Pl. 32; the other case followed an almost precisely similar course.

The most important feature in which great variations occurred has already been pointed out, namely, the independence in certain cases of the time of appearance of the gas-vacuoles from the programme carried out during righting. In some extreme cases the gas-vacuoles were very belated in their appearance; they became visible only after the righting process had been completed. Whenever this happened, they only reached at their maximum a very minute size, not more than 3 to 5μ, and very quickly disappeared again. In Experiment 122, for instance, the Arcella had turned over and half a minute later formed a gas-vacuole at 12; one minute later two other vacuoles appeared at 3 and 11; in another half-minute the vacuole at 11 had gone; half a minute later the vacuole at 12 disappeared; the one at 3 persisted for two minutes more, so that the whole time between the first appearance and the final disappearance of these vacuoles was only four minutes. This belated formation of gas-vacuoles will be referred to in the discussion of results (see p. 590).

B. Crushing Experiments.

Before dealing with the action of reagents on the living Arcella, a few experiments may be described which, although they did not help to decide the nature of the gas in the vacuole, nevertheless with the help of data which were brought out later, may add to the weight of evidence on one side. The object of these experiments was to isolate the minute gas-bubbles from the protoplasm, with the intention, if this were possible, of gradually collecting by some means or other a sufficient quantity of gas for analysis. Even if feasible, this would have been a very tedious process, as it would have meant the collection of the gas from a very large number of Arcellae. The method used in attempting to isolate the gas-vacuole was to start as in the standard experiment, and when the gas-vacuoles had reached or almost reached their maximum size a cover-glass with large wax feet was carefully placed over the drop of water. The wax feet were made of such a size that the space under the cover-glass was in depth three to four times the height of the Arcella. The cover-glass was first fixed to the slide by pressure over the feet with a needle, and then the centre of the cover-glass just over the Arcella was pressed down, bending the cover-glass by a quick sharp movement so as to suddenly crush the Arcella. Up to a certain point these experiments were successful, the gas was pressed out, and rose up to the cover-glass forming, however many vacuoles there had been in the Arcella, one single bubble. However, this bubble in all these experiments rapidly vanished, very much more rapidly than bubbles of oxygen of the same size in the same rain-water which were artificially formed under a cover-glass for the purpose of comparison. In order to account for this rapid disappearance, it was supposed that in crushing the Arcella a fragment of the protoplasm had adhered to the cover-glass, and that this absorbed the gas. This supposition was tested by adding neutral red to the rain-water in which the Arcella was crushed, and when this was done it was shown to be correct. In each case a portion of the protoplasm was found on the cover-glass in contact with the gas-bubble and stained bright red. As the bubble became smaller and smaller, it constantly remained in contact with the fragment of protoplasm, however minute this might be, until it finally disappeared, the last visible black speck indicating the position of the vanishing bubble was always on the fragment of protoplasm. It thus seemed evident that the gas was absorbed by the protoplasm itself, and not dissolved by the water. In three instances a gas-vacuole was observed which remained imbedded in a portion of the protoplasm after crushing. When this happened, the size of the vacuole remained constant for twenty (?) minutes, and then very gradually dwindled away until it reached such a size that the bubble appeared as a just perceptible spot of light, surrounded by a black ring. In this condition the bubble persisted, in one case it was watched at intervals and found to persist for more than twelve hours without alteration. This interesting abnormality in behaviour will be discussed when dealing with the general question of the nature of the gas in the vacuoles.

One crushing experiment gave a result which for a long time remained unexplained, and unexpectedly provided a complete explanation. The experiment was made by crushing an Arcella which had formed five large gas-vacuoles; it was crushed in the usual way, and it was found that the five bubbles pressed out did not coalesce to form a single bubble: in fact, two of them were caught inside the broken test; of the three which escaped, two in the course of the first minute after crushing were absorbed by protoplasm adhering to the cover-glass. The third, instead of disappearing, began rapidly to expand. It was measured by the eye-piece micrometer one minute after crushing; four minutes after crushing it had grown to 1⅔ times its diameter when first measured. It then steadily increased in size for 1 hr. 42 min., during which it was measured every few minutes, and found every time to be growing in diameter. At the end of this period it had grown to 5⅓ times its original diameter, its volume having increased, assuming that temperature and pressure had remained constant, to 455 times its original volume.

During the third hour after crushing, the large gas-bubble described above contracted slightly to four times its original diameter.

C. FORMATION OF GAS-VACUOLES UNDER ALTERED CONDITIONS.

The first experiments of this kind were made by modifying the conditions of chemical environment, and the changes made consisted in increase or decrease of the amount of the different gases normally present in water; the first series of experiments was on the influence of increased carbonic acid gas tension in the water.

(*a*) Increase of Partial Pressure of CO_2.—Bütschli implied, when he regarded the gas in the vacuoles as CO_2 that Arcella was immune to carbonic acid gas poisoning. If the gas in the vacuole were pure carbon dioxide, the pressure of the gas and the amount of it in the Arcella, considering its high solubility in water, must be far in excess of anything which can be resisted by ordinary protoplasm; in fact, the inference might be drawn that saturation with CO_2 would not kill Arcella. To test this, an active Arcella was placed in a hanging drop in a gas-chamber under the microscope, and pure CO_2 was run through the chamber. In four and a half minutes the contractile vacuoles had not only ceased their activity, but had disappeared, and the protoplasm had begun to protrude through the foramen. Within the next two or three minutes the protoplasm had swollen up and begun to assume a spherical shape, as it does in a dying Arcella, so that in about ten minutes after running in the gas, the Arcella was killed.

Further experiments with small amounts of CO_2 dissolved in water, from 2½ per cent. upwards, were made, with the result that Arcella was shown to be intolerant of quite small increases in the partial pressure of the dissolved carbonic acid gas.

(*b*) Absorption of Gas in Vacuoles by KOH Solution.—As these results all agree in making Bütschli's conclusion highly improbable, his method of treating the Arcella with caustic potash solution was tried on individuals containing gas-vacuoles. The first experiment was made with a freshly made strong solution, about 10 per cent. of caustic potash in water. The gas in the vacuoles began to disappear in about one minute, and in three minutes had all been absorbed. The same solution was then saturated with air by shaking, and another Arcella containing large vacuoles was treated with the solution. The first result of the killing of the protoplasm was that the large and irregular gas-vacuoles became spherical, the two smallest vacuoles, which were 10μ in diameter, were not dissolved for three minutes, and the largest vacuole, which was 60μ long, was not dissolved for six minutes. It is quite clear from these experiments that the gas was not CO_2, as the rate of solution was quite plainly dependent on the amount of air dissolved in the caustic potash solution. In the first experiment; the air in the water used for dissolving the potassium hydrate was, to some extent, expelled by the heat generated as the hydrate went into solution, and the gas in the vacuole was seen to dissolve quickly. When the air was replaced by shaking, the gas in the vacuole dissolved more slowly in the solution. If this experiment gives any clue at all to the nature of the gas in the vacuole, it indicates that it must contain one or more of the gases which occur in air.

(*c*) Decrease of Partial Pressure of Oxygen.—The first experiments were made by the simplest possible means, namely, by breathing expired air into the gas-chamber where Arcellae were living in the hanging drop. The Arcellae always reacted to this by the formation of gas-vacuoles, and in order to determine whether this reaction was due to the reduced partial pressure of oxygen or to some other alteration in the composition of the air from the lungs, the following experiments were carried out.

In the first place the atmospheric pressure of the gas in the air-chamber was reduced by two-thirds, by simply sucking out air by the mouth. The two Arcellae in the hanging drop both formed gas-vacuoles within a few seconds after the pressure had been reduced.

In the next experiment the atmospheric pressure remained constant, and the air supplied to the gas-chamber contained oxygen at the reduced partial pressure of 9·94 per cent.[1] It contained no carbonic acid gas. The first result of passing in this air was greatly increased activity of the external pseudopodia, and this was quickly followed by gas-vacuole formation sufficient in a few minutes to float the Arcella up to the cover-glass.

A series of experiments was then tried in which Arcella was placed in water, in which the partial pressure of the dissolved oxygen had been reduced. This was done by taking water from the bottom of a ripened culture of Paramecium in hay infusion. This water was seen from the behaviour (reduction of movement) of the motile organisms in it to be completely or almost completely without oxygen. The effect of this water on Arcella was immediate, in the formation at once of gas-vacuoles. The infusion water was found to be acid, and as the stimulus to form gas-vacuoles might possibly be attributed to the presence of acid, the effect on Arcella was tried of weak organic acids of about the same strength as the infusion, measured by its effect on litmus paper. The acids used were acetic acid, formic acid, citric acid, and carbonic acid gas. In none of the numerous experiments did the Arcella respond by forming gas-vacuoles.

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[1] I have to thank Dr. C. G. Douglas for kindly preparing the air and for determining its oxygen pressure.

Another series of experiments was made with water from which the air had been expelled by boiling and kept out to some extent by dissolving in the hot water some cherry gum or cerasin; Arcella when placed in this solution responded by forming gas-vacuoles as long as the solution was fresh. After standing several days the solution no longer gave this result; several Arcellae placed in it formed no gas-vacuoles.

The result of these experiments testing by varied means the influence of reduced partial pressure of oxygen, is that however the reduction is brought about, the response in forming gas-vacuoles is the same. In fact, in none of the experiments in which these different means of reduction of oxygen pressure were applied to Arcella did it ever fail to bring about a response.

These uniformly positive results strongly support the view, which has long been held, that the function of the gas-vacuoles is to reduce the specific gravity of the Arcella, and float it up to the surface which is rich in dissolved oxygen. But it is now possible to go a step farther. In considering the natural causes, or changes in the environment, which might possibly stimulate gas formation for hydrostatic purposes in Arcella, the first consideration was, what is the most obvious and most important physiological difference between the water at the bottom and the water under the surface-film? This is clearly a difference in oxygen pressure. The analyses of pond-water for the determination of dissolved gases carried out by Knauthe (1898, 1899) and Zuntz (1900) show that pond-water may, by the influence of physical and biological conditions, be entirely deprived of oxygen. Owing to the slow rate of diffusion of oxygen in water, the bottom water of a pond or ditch, exhausted of oxygen, will be replenished only a long time after the surface layers. Hence it will clearly benefit those aerobic or semi-anaerobic organisms which live on the bottom, to have a means of escape which will rapidly carry them from a level of oxygen depletion to a level of oxygen plenty. The principal stimulus to form gas-vacuoles in Arcella and similar organisms which live at the bottom of ponds and ditches, is lack of oxygen. There may be, and probably are, other sets of external conditions which stimulate the production of gas by these organisms, and there are also conditions arising within the cell which stimulate the gas-forming structures. These will be described and discussed later.

CHAPTER V.—EXPERIMENTS WITH VITAL STAINS.

A. METHYLENE BLUE.

THE theory of methylene-blue staining in living tissues generally adopted is that proposed by P. Ehrlich (1885), that the stain is taken up into the cell or the tissue, is there reduced to leuco-methylene blue and becomes colourless, and that the colour returns when the cell or tissue is the seat of free oxygen production which brings about the oxidation of the leuco-compound turning it back into methylene blue.

Assuming that this theory is, so far as it goes, a true statement of what occurs when methylene blue is taken up by the living cell, experiments were made with Arcella put into methylene-blue solution, and then stimulated to evolve gas.

(*a*) Reversal Experiments.—The method of these experiments was as follows. An Arcella was put on a slide in a drop of rain-water, obverse side uppermost, and when the Arcella had attached itself to the slide by its pseudopodia the rain-water was drawn off until the Arcella was almost left dry, and a drop of methylene-blue solution in rain-water was added. No cover-glass was used in this experiment. The strength of the methylene-blue solution varied between 1 in 20,000 and 1 in about 500,000. After five minutes, at least, or at most twenty minutes, the methylene-blue solution was removed, and the Arcella washed in several changes of rain-water. It was then examined under the microscope, and after this treatment the protoplasm was found to be perfectly colourless. When the Arcella had been left in the methylene blue for more than twenty minutes, or in a stronger solution than 1 in 20,000, a certain amount of blue staining might occur in the protoplasm, but after washing, if present, it was soon reduced and was never a deep stain. In later experiments, the Arcella was never left in the methylene-blue solution for more than five to ten minutes, as, after this time, the protoplasm always remained colourless, and the Arcella could then be treated further. The next step was to reverse the Arcella with a needle, and watch it under the microscope.[1]

When reversed, the behaviour of the Arcella is the same as under the conditions of the standard experiment. The external pseudopodia are protruded, but instead of being colourless, they are just perceptibly blue. Within a minute or two a blue patch appears in the marginal zone, close to the edge, and is followed rapidly by the appearance of four or five similar patches, the combined area of which gradually covers the whole of the outer zone, the spreading process being completed in three to five minutes. The methylene blue has obviously been taken up by the protoplasm and reduced as fast as it is absorbed, and when the gas which forms the vacuoles begins to be evolved the leuco-methylene blue is immediately oxidized and the colour restored. The above is what happens when the blue stain appears slowly. In cases where the stain develops more rapidly, it suddenly appears, within one minute after reversal, round the whole of the edge of the marginal zone, and within a few seconds spreads inwards as far as the edge of the central mass. If a young daughter Arcella is being observed, the marginal pseudopodia soon appear, and are more evident than they were in the standard experiment, being distinctly blue of a far deeper shade than that of the external pseudopodia. Gas-vacuoles then form, either before or after righting, they become as large or larger than any seen in the standard experiments (fig. 8, Pl. 34). While they are growing, a glance at the nuclei shows that the karyosome and the radiating filaments have turned a pale blue; the cytoplasm is distinctly blue in the outer zone, but hardly appears to be blue in the central mass. If this central cytoplasm, however, is examined under the highest available power, there is seen to be a very faintly stained, coarse blue network. The appearances just described are all shown in fig. 8, Pl. 34. The large gas-vacuole in this figure is the largest I have seen. The two smaller gas-vacuoles in this figure are drawn in the act of expanding. It is interesting to note that during expansion a well-marked radial structure of the cytoplasm (see fig. 8, Pl. 34) is made evident by more deeply stained radiating bands spreading out from the vacuole as a centre. The bands sometimes branch, and these branches may anastomose with those from neighbouring bands. The gas-vacuoles reach their maximum size without any check; the Arcella rights itself, if it has not already done so, and then the vacuoles begin to diminish. Towards the end of this phase in each methylene-blue experiment, a state of things is observed which was never seen in the standard experiments. The peculiarity consists in the behaviour of every gas-vacuole just before disappearing; it is illustrated in fig. 10, Pl. 35. When each of the gas-vacuoles reaches a size shown in fig. 10 B, Pl. 35, instead of continuing to shrink, it expands until it reaches the size shown in fig. 10 A, Pl. 35. It then shrinks to the size shown in fig. 10 B, Pl. 35; then it expands again as in fig. 10 C and D, Pl. 35; shrinks again, and again expands; shrinks, expands, shrinks, expands, shrinks and at last disappears, as shown in the series of fig. 10 E to M, Pl. 35. This alternate shrinking and expanding may take place as often as nine times; during these alternate changes in size, a series of corresponding alternations in depth of colour takes place in the blue protoplasm surrounding each disappearing vacuole. Every time that the vacuole expands, the surrounding cytoplasm becomes of a paler blue, and each time the vacuole shrinks, the surrounding cytoplasm turns a deeper blue, deeper in fact than has been observed under any other circumstances. This is clearly shown in fig. 10 B, E, H, J, and L, Pl. 35, where the radiating structure is here seen again, this time not so distinctly or not at all in connexion with the expanding vacuole, but very well-marked around the dwindling and the disappearing vacuoles. When the vacuole finally disappears, it leaves at the spot where it has vanished a clear, colourless minute area of hyaline protoplasm (fig. 10 M, Pl. 35). This spot is always in the marginal zone and either in, or close to, the base of a marginal pseudopodium, which at the moment of disappearance and for about thirty seconds to a minute afterwards is stained a deep blue, as in fig. 10 M, Pl. 35, the colour extending for a short distance into the protoplasm round its base. Two minutes after the vacuole is gone the marginal pseudopodia in the neighbourhood have all become pale (fig. 10 N, Pl. 35); they subside in the ordinary way, and after the last vacuole has vanished, the whole of the protoplasm gradually loses its colour until in twenty minutes to half an hour there is no more visible trace left of the methylene blue, it has all been reduced once more. If the Arcella is now again reversed, the blue colour reappears, and the whole process of gas-vacuole formation, with the accompanying colour changes described above, will be repeated in a similar way. In several experiments the Arcella was replaced alone in a culture-glass, and reversed under the microscope twenty-four hours later; the methylene blue reappeared in the colourless protoplasm apparently without any diminution of intensity since the reversal on the day before, showing that the methylene blue had in the interval neither been excreted nor washed out of the protoplasm.

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[1] The most useful combination for this purpose was found to be an 8 mm. apochromatic objective with oculars 6 to 18, as this gave high magnification, when wanted, with great working distance between the uncovered drop and the objective.

The abnormal behaviour of the gas-vacuole, just before its disappearance in the methylene blue Arcella, was constant in all the individuals treated with this stain. In the standard experiments of the several hundred cases in which a gas-vacuole was watched as it vanished not one deviated from the regular course of steady and constant diminution, in no single case was there any sign of the reversion which is of constant occurrence in the methylene-blue experiments. The only case of reversion in the standard experiments is that already described and figured, and one other where it took place during the expansion phase of the gas-vacuole. Other experiments, to be described later, point to the importance of these disturbances in the normal life of the gas-vacuole in forming a hypothesis on the method of gas production.

(*b*) Reduced Partial Pressure of Oxygen.—An Arcella containing leuco-methylene blue is obviously in a condition to indicate, by the production of methylene-blue stain in its protoplasm, any reaction of the oxidase system to an external stimulus. A whole series of experiments on the action of such stimuli has been planned and partly carried out, but one experiment only will be given here.

This experiment was carried out in a similar way to that just mentioned, in the same gas-chamber with hanging-drop, but the Arcella was allowed to settle on the cover-glass and was not on the surface film of the drop. The cover-glass was used as a slide while the Arcella was being treated with methylene blue. The strength of methylene-blue solution was 1 in 1,000,000 and the Arcella remained in this for only one minute before the staining solution was washed off with rain-water. When examined ten minutes after this, the protoplasm was colourless. The cover-glass was then inverted, and placed on the gas-chamber and sealed down. One of the tubes to the cell was closed, and the other connected by india-rubber tubing to a glass tube standing in glycerine, so that the atmospheric pressure in the gas-chamber would remain unchanged throughout the experiment while the oxygen partial pressure was decreasing. The oxygen was absorbed by a method which enabled the exact time to be noted at which the absorption commenced, and also insured that the absorption would be slow and gradual. This method which proved easy to carry out was a modification of the pyrogallol and caustic potash method. A small block of plasticine was placed at one side of the gas-chamber on its floor, just large enough to take side by side two depressions made with the rounded end of a glass stirring rod, and these depressions were joined up by a broad notch in the plasticine. In one depression was put a large drop of 10 per cent. caustic potash solution, and in the other a small heap, about the same size as the drop of caustic potash, of crystallized (not sublimed) pyrogallol. When everything is arranged for the observation to begin the slide with the gas-chamber is tilted so that the drop of potash solution runs down over the pyrogallol and dissolves it. The absorption of oxygen is then started and this moment is noted as zero time for the experiment.

At the end of five minutes the glycerine in the gauge had risen according to rough calibration 0·1 c.cm. (the total volume of air in the chamber and tube was about 1·1 c.cm.), and the protoplasm of the Arcella had already begun to show a faint blue tinge, in another minute the protoplasm was distinctly blue. The next ten to twelve minutes were occupied by the protoplasm in ejecting remains of digested food such as diatom tests. After this the marginal pseudopodia began to be active and continued in lively movement at two points in a way which suggested the impending formation of two gas-vacuoles; however, at the end of about fifteen minutes, the movements had subsided and no gas-vacuoles had formed. The blue stain in the protoplasm had, however, now become still darker and the karyosomes were now blue. The glycerine continued to rise in the gauge for six and a half hours, so that the oxygen was not completely absorbed until some time later than this. The Arcella was kept under observation during the whole time, and its behaviour was unchanged as regards the movements and the retention of the methylene-blue stain, which at no time was lost and in fact became deeper and deeper as the experiment went on. The marginal pseudopodia were constantly formed and retracted, and occasionally marginal lobopodia were put out. Whenever one of these with its filopodia reached its last stage before disappearing it became much more deeply stained, just as it would have done if a gas-vacuole had been present. From the beginning to the end of the experiment the Arcella never ceased its crawling movements, but towards the end of the experiment from 6 hr. 30 min. to 8 hr. 10 min. the movements became extremely slow and finally almost stopped. At this point air was allowed to enter the gas-chamber and during the next fifteen minutes the cytoplasm lost considerably in depth of stain; for another hour the methylene blue became paler and paler; an hour later there was not much change, the protoplasm was still pale blue. It was observed during these two hours that the contractile vacuoles were discharging methylene blue which they had not done during the time when under low oxygen pressure. The loss of colour in the protoplasm may be partly accounted for by this discharge from the contractile vacuoles.

(*c*) Toxic Action of Methylene Blue.—An Arcella was placed at zero time into a very weak solution (1 in 20,000 to 50,000) of methylene-blue BX. Although it lay on the slide with its foramen down, and although it was not reversed with a needle, it formed five gas-vacuoles in four minutes, and later produced two more. During the next forty-five and a half minutes these vacuoles behaved just as they did in other specimens treated with methylene blue. Before disappearing they expanded and contracted alternately five times. This experiment shows that the Arcella was a normal specimen. When the vacuoles had vanished, the protoplasm became colourless except that a faint blue tinge could be seen in the ectoplasm of the pseudopodia, and the karyosomes remained blue. At 1 hr. 42 min. the greater part of the protoplasm was protruded from the foramen in a rounded mass, and the whole of the protoplasm was now quite dark blue, and the karyosomes still darker. Contractile vacuoles were present, but remained in diastole. At 4 hr. 7 min. the condition was unchanged, except that the protruded protoplasm had become spherical. At 4 hr. 47 min. the protoplasm was still more protruded from the foramen, and had become bilobed, as though two blunt lobopods had formed, with four contractile vacuoles in one lobe, and none in the other. At 6 hr. 42 min. it was found that the protoplasm had abandoned the test, and was crawling about actively as a mass of naked protoplasm, with the characteristic movement of Amoeba limax. Both nuclei were present, but there was only one gigantic contractile vacuole, about three times the diameter of the nucleus, and situated on one side of the base of the big lobopod. The whole of the cytoplasm and the karysomes were stained bright blue. At 7 hr. 2 min. it was observed that the protoplasm constantly changed the direction of its streaming movements, so that the Arcella remained on the same spot. Two enormous contractile vacuoles were now present. At 7 hr. 42 min. the pseudopodia disappeared, so that the protoplasm formed a spherical mass, and a third large contractile vacuole was formed. At 7 hr. 49 min. a lobopod was again formed, and movement without progression was continued as before by protruding a short lobopod as broad as the diameter of the sphere in a sluggish manner and in different directions, with the result that the protoplasm did not move away from the spot where it was lying. The empty test was carefully pushed into contact with the protoplasm, which showed no sign of being stimulated to return to it. When observation stopped at 10 hr. 33 min. the cell had become motionless and spherical, and the two nuclei were lying close together at the bottom of the sphere.

B. Toluidine Blue.

As might be expected the results obtained with Toluidine blue agree very closely with the methylene blue results. This stain is not quite so satisfactory as methylene blue, because the reduction in the animal does not leave the protoplasm without any tinge of colour. An Arcella was treated with a solution of toluidine blue so weak that there was no colour at all visible in a deep watch glass full of solution placed on white paper, the strength would be in the neighbourhood of 1 in 1,000,000. The Arcella was left in this solution for twenty-three minutes. The stain was not reduced, weak as it was, by the protoplasm, and different parts were stained in different ways, a blue network appeared in the apical region like that in the methylene-blue specimen (fig. 8, Pl. 34), the refringent bodies in the protoplasm were stained greenish blue and the karyosomes pale blue. This colour persisted for six hours in rain-water, in fact at the end of this time it had spread, as not only the refringent bodies, but the whole of the cytoplasm had become pale greenish blue. After reversal the external pseudopodia when extruded were pale blue. A gas-vacuole appeared very soon after the blue tinge had spread over the outer zone just as it did in methylene-blue experiments. The Arcella soon righted itself and when it had turned over a gas-vacuole was found. It was diminishing quickly, but two minutes later it was expanding, in another minute it was shrinking and a deep blue halo had appeared round it, while all the remaining cytoplasm, at this time, contained no blue. In the next two minutes the vacuole enlarged and the blue halo faded away, then the vacuole decreased for three minutes and the halo reappeared. During the next minute the vacuole enlarged slightly, but by this time it was so small that it could pass out into the base of a marginal pseudopodium which turned blue, two minutes afterwards the shrinking had continued and the internal pseudopodium with the filopodia on it had turned dark blue ; in another minute the gas-vacuole had grown larger and then the experiment was ended by the specimen being killed in sublimate. In the space of thirteen minutes the gas-vacuole had alternately diminished and increased in size four times, and each time a corresponding appearance and disappearance of blue colour took place in the cytoplasm surrounding it.

C. Trypan Blue.

An Arcella was treated with a solution of trypan blue so weak that the colour in a watch glass standing on white paper was just discernible, it remained in this for twelve minutes. Much colour was taken up and both the whole of the cytoplasm and the karyosomes retained the stain for some hours. The last traces of blue in the cytoplasm were seen in the marginal zone, and the cytoplasm did not become quite colourless until nearly four hours after washing off the solution of stain. A few minutes later the Arcella was reversed and the marginal zone was found to be tinged with blue immediately. Five minutes later the first external pseudopodium was extruded and was pale blue. The karyosomes had also become pale blue, and the cytoplasm became blue in the same way as it did in methylene-blue experiments. Five gas-vacuoles appeared before the Arcella righted itself. Three minutes after righting all five vacuoles were still expanding, but one minute later still they all had begun to contract, and a blue halo had appeared round each of them. The contraction continued in this Arcella without a break until all the gas-vacuoles had disappeared; ten minutes after, it had righted itself. While the contraction is going on, a blue halo persists round each vacuole, this halo became smaller rapidly as the vacuole dwindled away until the final stage when the vacuole was a small black speck. When this speck has gone a hyaline patch of cytoplasm coloured blue was left behind and the blue patch faded away in three to four minutes, losing its colour and being invaded by granules from the surrounding cytoplasm. In another trypan blue Arcella, of the six gas-vacuoles which began to disappear after righting, five began to expand (one had already vanished) simultaneously for less than one minute just before they all shrank and became occluded. In the trypan-blue experiments the whole of the cytoplasm became colourless at the time when all the vacuoles started contracting, the blue halos appeared round the vacuoles immediately afterwards when the contraction had proceeded for about one minute.

D. Malachite Green.

This basic rosaniline salt has been used by bacteriologists to stain the culture medium in order to indicate areas of reduction and oxidation. This suggested the possibility of the reduction of malachite green in the living cell, and it was used as a vital stain on Arcella with complete success. The solution used was, as usual, extremely weak. It was applied to an Arcella found, under the high magnification, to be already reversed when transferred to the slide, and to have formed a gas-vacuole. This gas-vacuole, and another which began to expand ten minutes later, behaved in one respect as gas-vacuoles never did in any other experiment. Before the Arcella righted itself the two vacuoles expanded and contracted alternately five times and the two vacuoles did not, as they generally do, act in unison, but while one was expanding the other was contracting. After righting (with one interruption shortly before disappearing), when for about thirty seconds both vacuoles expanded, they steadily decreased in size for eight minutes and disappeared.

All the colour phenomena seen in the methylene-blue experiments were repeated with the same if not greater distinctness in malachite green Arcellae. The living protoplasm does not seem to be seriously affected by the stain, although the disturbance of the gas-vacuole formation was more marked with this than with any other vital stain used. An Arcella treated with malachite green used for reversal experiments on one day, reacted on the next day, twenty-two hours later, just as it had done the day before. In this interval the stain had been completely reduced to the leuco-compound. In the alternate contraction and expansion of the gas-vacuoles described above, a green halo was formed in each case round the contracting vacuoles, and disappeared round the expanding vacuoles. As the vacuoles were disappearing the protoplasm round them became bright green, and where they disappeared a bright green patch was left which persisted for two or three minutes. After the Arcella had righted itself the protoplasm was examined for stain and it was found that a green reticulum was present in the outer zone but not in the central mass. When the gas-vacuoles had disappeared, for five minutes the whole of the cytoplasm was found to contain a stained reticulum which was now of a more bluish green. The network corresponded to that drawn in fig. 8, Pl. 34 in the central mass. At this stage the karyosomes were stained the same colour as the network but slightly paler; during the persistence of the vacuoles the karyosomes were distinctly green, not bluish green. The appearance of a dark green edge round the marginal zone just after reversal was very striking and the external and marginal pseudopodia were coloured, the latter quite deeply. In the Arcella with leucomalachite green the first elements which showed colour after reversal were the refringent bodies, and at a later stage these bodies showed beautiful dichroic effects in green and brown.

E. INDIGO-CARMINE.

The general results with this vital stain (sulphindigotate of soda) were the same with regard to the appearances connected with the gas-vacuoles as in the methylene-blue experiments. The colour assumed by the cytoplasm in the gas-vacuole reactions was invariably blue. The halos, the patch seen where each vacuole disappeared, the marginal zone, the external and marginal pseudopodia all gave the same reactions as they did with the stains already described. The reactions just mentioned all belong to those which take place after reversal. In one Arcella before reversal the whole of the cytoplasm, with the exception of the contractile vacuoles, was pink and did not turn blue until five or six minutes after reversal. The karyosomes which from the beginning were stained pink still remained pink for twenty minutes after reversal. The rays from the karyosomes were also pink. In this Arcella, the behaviour of the karyosomes observed after the animal had been reversed and had righted itself for the second time was very remarkable. About ten minutes after the gas-vacuoles had gone the karyosomes were pink, with the exception of their outer surface which was blue, giving them the appearance of being enclosed in a blue shell; in another eight minutes the karyosomes had turned blue, but were now enclosed in a pink shell. This Arcella was again turned over twelve minutes later, and when the karyosomes were then examined the colouring had again been changed, they were now pink internally with a blue surface layer. Reduction of the indigo-carmine appears to take place, as the protoplasm, after the vacuoles have vanished, becomes almost colourless. Reduction was not observed before reversal in one Arcella which was watched for twenty minutes after being taken from the staining solution. The Arcella was then reversed because it was observed that the indigo-carmine was being excreted. The fluid in the contractile vacuoles was blue and stood out distinctly against the pink cytoplasm. In this experiment, helped perhaps by this contrast, an observation was made which was new and has not been repeated since, namely, that the contractile vacuoles are fed by radiating channels in the surrounding cytoplasm like those commonly observed in some Ciliates (e. g. Paramecium).

F. BRILLIANT CRESYL BLUE.

The strength of solution applied to the Arcella was just sufficient to allow the blue to be made out in a drop on a slide over white paper. In this the Arcella was left for seven minutes and then put into rain-water. No observation was made until four hours later, then the protoplasm was found to be quite colourless; when reversed, no colour appeared and the Arcella righted itself after fifteen minutes without forming gas-vacuoles and without producing any sign of stain. The same Arcella was reversed five hours later, that is nine hours after removal from the solution of stain. Just after reversal the protoplasm was entirely colourless with the exception of a faint violet tinge in some fluid vacuoles. This individual had a large number of highly refringent colourless spherical bodies in the cytoplasm. The first colour change, apart from the fluid-vacuoles, was seen in the karyosomes which were pale violet thirteen minutes after reversal; by this time the colour of the fluid vacuoles had become much darker. The first two gas-vacuoles appeared fifteen minutes after reversal, and four minutes later three others were present and all five enlarging. The cytoplasm round them had a yellowish appearance, and only when two of the vacuoles began to contract did the green colour appear in the cytoplasm. Half an hour after reversal the Arcella had begun to right itself, but was still only slightly tilted; the gas-vacuoles were extremely large and the karyosomes which had been violet now became green. At the same time the refringent bodies had turned green of a bright shade like the stain of methyl green. Forty minutes after reversal the gas-vacuoles began to contract in unison, and the cytoplasm turned a vivid green which extended into the hyaline protoplasm of the pseudopodia and more especially the marginal pseudopodia. Forty-five minutes after reversal the Arcella had righted itself and the gas-vacuoles were found to have grown larger, while the green stain in the cytoplasm had gone, leaving only a faint green stain in the karyosomes and in the refringent bodies. During the next eighteen minutes the gas-vacuoles gradually dwindled away until they disappeared, the cytoplasm becoming of a brilliant green colour around them. When the gas-vacuoles finally vanished they left behind a distinct green patch, which only persisted for about fifteen seconds. Half an hour after righting, and about fifteen minutes after the last gas-vacuole had gone, the colour had all gone from the cytoplasm leaving only a very faint green stain in the refringent bodies. During the preceding ten minutes it was observed that the contractile vacuoles had their contents stained green.

G. Haematoxylin.

The experiments with indigo-carmine and brilliant cresyl blue pointed to the possibility of using other vital stains which might confirm or add to the evidence which these stains gave. It was thought possible that the living protoplasm might take up haematoxylin, and then if the gas production involved oxidation, the haematoxylin would be converted into a coloured salt of haematein.

A very weak solution of haematoxylin in rain-water (one minute crystal in a watch-glass full) was applied to an Arcella. Examined seven minutes later before reversal, the karyosomes were found to be pink and the reticulum blue, three minutes later the karyosomes had become blue and after reversal they remained blue. Several individuals were experimented on and reversed a number of times giving in every case similar results which were, with certain modifications, a repetition of the results obtained with methylene blue, indigo-carmine, malachite green, and brilliant cresyl blue. With the exception of the transitory pink stain in the karyosomes no red reaction appeared in the haematoxylin experiments, that is to say the only indication of red appeared in the nucleus and not in the cytoplasm. The Arcella in each reversal experiment was turned over soon after the application of the stain, and no observation was made showing the reduction to a colourless compound corresponding to the leuco-base of methylene blue, malachite green, &c. All the phenomena observed during the contraction of the gas-vacuoles with other vital stains were repeated in the haematoxylin experiments so far as the vacuoles themselves were concerned. In one experiment alternate diminishing and enlarging took place six times after righting. In this experiment the modifications of the changes in colour mentioned above were very marked. The effects observed in the experiments with other vital stains were, in the haematoxylin Arcella, exactly reversed in the immediate neighbourhood of the dwindling vacuole. Instead of the colour becoming deeper and forming a halo round the vacuole as it contracted, the surrounding cytoplasm became pale and the blue halo appeared when the gas-vacuole expanded. When the gas-vacuole finally vanished, the clear patch left behind, instead of being more deeply coloured than the rest of the protoplasm was either much paler or completely bleached. This result did not spread beyond the area of the halo, outside this the cytoplasm remained blue and even the marginal pseudopodia were not always bleached when the vacuole at their base was contracting. When, however, the expanding vacuole was minute and reaching the end of its existence it was very close to the base of the marginal pseudopodia, and then they also were bleached. In one experiment where three gas-vacuoles had been formed and disappeared in unison very rapidly, not only did the colour disappear from the cytoplasm near the vacuole but the whole of the cytoplasm was bleached. Five minutes later the outer zone on its upper surface was covered with a pale blue reticulum which was not continued over the central mass. This slight stain persisted until the Arcella was reversed, about twenty minutes later, and then the stain became deeper, especially in the marginal zone. Except for the difference in colour there was no deviation from the appearances in the cytoplasm obtained with the other vital stains; although the colour phenomena around the vacuoles were exactly opposite to those seen in methylene-blue specimens.

H. NEUTRAL RED.

Although the action of indicators in the presence of protein is very uncertain, this dye has been much used by older observers before the theory of indicators was formulated. As the above experiments, especially those with indigo-carmine, gave colour reactions which pointed to probable changes of H-ion concentration, it was thought worth while to see whether the range of H-ion concentration changes might lie within the range of neutral red as an indicator. More particularly it was hoped that the clear patch, left in the cytoplasm behind each gas-vacuole as it vanished, would give some colour reaction showing that the activity of gas absorption was accompanied by displacements of the H-ions.

To a certain extent the results obtained confirmed the results with other vital stains, but the faintness of all the neutral red reactions made these results less striking. However, the colour effects can be considerably increased by examining the Arcella with transmitted light of an approximately complementary colour, say a blue green, by using a tinted glass on the substage.

As soon as the Arcella had been washed in rain-water and put under observation, it was found that the karyosomes had been stained and become pink. The contents of food vacuoles were either stained a bright pink or distinctly yellow, showing that in Arcella the reaction of the digestive fluid is not sufficiently acid to act quickly on the newly ingested food. While the gas-vacuoles were growing larger, the surrounding cytoplasm became darker than the rest of outer zone. The usual alternations during the contraction phase were seen with this vital stain, and one of the gas-vacuoles enlarged and contracted six times before it disappeared. As it was enlarging for the sixth and last time the halo was not pink but yellow. The clear area left where the vacuole had gone was pink and became deeper in colour for half a minute after the disappearance of the vacuole, in another half-minute the clear area became colourless. At this stage the whole of the cytoplasm was stained deep pink, while the karyosomes were still faintly tinged with pink. At the beginning of the experiment just described a small gas-vacuole formed and disappeared two minutes later. About three minutes after its disappearance the second vacuole was growing larger and had a distinct pink halo; at this time the area occupied by the vanished gas-vacuole became deeper pink but the gas-vacuole did not reappear.

Careful observations on the contractile vacuoles showed that the reaction of the fluid contained in them has an H-ion concentration not sufficiently high to turn the Neutral Red yellow, the contents of these vacuoles were distinctly pink.

I. The Influence of Vital Stain on the Gas-vacuoles.

The behaviour of the gas-vacuoles in an Arcella containing vital stain differs so widely from that seen in the standard experiment that the vital stain is clearly a disturbing factor. In the methylene-blue experiments the dwindling of the vacuole in its last phase is interrupted several times by an interval of growth, accompanied by corresponding changes in the colour of the protoplasm. These changes in colour are such as to show that the methylene blue acts as though it were an addition to the substrate whatever the latter may be. In the standard experiment the oxygen in the dwindling vacuole is transferred by the oxidase to some substance or substances in the protoplasm which must be unsaturated and ready to take up the whole of the oxygen supplied. When methylene blue is present and some of it as a leuco-base, a part of the oxygen is diverted to the oxidation of the extraneous substrate, viz. the leuco-methylene blue. The result is that some of the colour in the methylene blue returns each time the gas-vacuole shrinks. At every reversal of the process the methylene blue is reduced to the leuco-base.

CHAPTER VI.—ENGELMANN'S OXYGEN TEST.

An enormous pressure of gas is required to start the formation of a gas-bubble, and before this pressure is reached the solution of gas in the protoplasm must reach saturation point. The great rapidity with which the gas-vacuoles sometimes expand led to the expectation that a certain, probably very small, amount of gas would slowly diffuse out into the water from an Arcella with gas-vacuoles, if the gas is oxygen. The delicate biological test for oxygen of Engelmann seemed to be exactly adapted to indicate the nature of the gas. Some observations already made by accident during the reversal experiments might have been interpreted as successful Engelmann experiments. Several times the drop of water in which an Arcella was lying reversed contained a number of minute free swimming Flagellates. When the external pseudopodia had been extruded and were either waving about or groping for a hold on something solid, it was seen that all the Flagellates in the drop collected above and among the pseudopodia. The drop was searched with black ground illumination to show up any Flagellates which might be scattered in it, and none were found away from the Arcella. Experiments were made to test whether the attraction of the Flagellates to the Arcella was due to positive chemotactic action on the Flagellates by oxygen escaping from the pseudopodia.

The organism used to test for the presence of oxygen was the Flagellate obtained in an infusion of one edible pea in 250 c.cm. of rain-water. At the end of ten days a Flagellate resembling Peranema was found in quantities in the infusion. A drop taken from the surface contained active Peranemas but the infusion at the bottom of the culture-flask contained only perfectly motionless individuals. A drop containing these was put on a slide, and watched for some time in order to see whether they would begin to move about when the drop had been exposed to the air. At the end of ten minutes they were still motionless and an Arcella, after having been washed in a little of the infusion fluid from the bottom of the flask, was placed in the drop.

The Arcella was reversed and soon put out external pseudopodia. When one of the pseudopodia passed over the edge of the test and down towards the surface of the slide as shown in fig. 1 A, Pl. 30, the tip of the pseudopodium was focussed and kept in focus as it moved down to the slide. When the tip of the pseudopodium rested on the slide and a Peranema lying motionless at a distance of not more than 10 μ was approached by the pseudopodium, the flagellum of the Peranema began to beat freely. When the pseudopodium remained at a distance of 10 μ or less from a Peranema for a quarter or half a minute, the movement of the Flagellate became slightly more vigorous, so that it sometimes shifted its position while still lying on the slide. During the experiment none of the Flagellates lying away from the Arcella began to move, so that the movements observed could without any doubt be put down to the influence of the pseudopodium. The motion of the flagellum in Peranema ceased within one second of the withdrawal of the pseudopodium.

Peranema and similar Flagellates have been used by botanists for demonstrating the evolution of oxygen from Algae, and there seems to be no doubt that the movements in these experiments are brought about by oxygen and not by any other substance given off by the Algae. The conclusion must be drawn from the experiment that oxygen is given off from the active pseudopodia of Arcella in exceedingly minute quantities. Accepting Engelmann's measurements of the quantities of oxygen required to produce such movements, it would appear that the amount of oxygen given off from the tip of the pseudopodium in about one second is of the magnitude of one-millionth of a milligram.

CHAPTER VII.—TESTS FOR INTRACELLULAR OXIDASES AND CATALASES.[1]

The following consideration pointed to the possibility that the use of oxidase reagents would give some positive results in the cytoplasm of Arcella: (1) The exclusion of gases which might be poisonous and those which are not known to be given off at all, or not given off sufficiently rapidly by protoplasm, leaves oxygen as the only gas which may be given off. It has hitherto not been definitely shown to be secreted by animal protoplasm, with the possible exception of cells of the 'red gland' in the air-bladder of fishes. (2) The wide distribution of intracellular oxidases, peroxidases, and catalases in metazoan cells. (3) The production of oxygen during the activity of the external pseudopodia as demonstrated by the application of Engelmann's test. (4) The stimulation of some element in the cytoplasm resulting in oxygen secretion by reducing the partial pressure of oxygen in the surrounding medium.

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[1] The term Oxidase throughout this paper is used in its widest sense to include not only the oxidases proper but also peroxidases and oxygenases, except where it is obvious from the context that the restricted sense is intended.

A. Indophenol Reaction.

This test was applied to Arcella after killing either with formaldehyde solution or with corrosive sublimate, and in both cases similar results were obtained. Kastle found that the action of oxidases is not impaired by treatment with formaldehyde, but according to Vernon, Bokorny has shown that sublimate acts as a poison on enzymes just as it does on living organisms. As will be seen from the results of the following experiments, the poisoning action under the conditions to be described does not take place in a widely distributed group of oxidases.

The method of applying this oxidase reagent to Arcella is very simple. After killing with 4 per cent. formaldehyde solution the Arcella is washed in three or four changes of distilled water and the indophenol reagents are then applied immediately. The strength of the solution was M/100 of indophenol reagents in 0·25 per cent. of carbonate of soda and was used freshly made. The Arcella in these experiments was always reversed and allowed to form gas-vacuoles just before it was killed, in order to apply the test when the oxidase, if present, was at the height of its activity. The specimens killed with sublimate usually, and those killed with formaldehyde sometimes, retained the gas in the vacuoles when preserved. As soon as the indophenol solution was applied to an Arcella with gas-vacuoles, the gas immediately disappeared, and simultaneously the blue reaction commenced to show itself, not only in the neighbourhood of the gas-vacuoles but also round the whole outer zone. The stain continued to become deeper for half an hour. Each of the spaces in the marginal zone is the cavity occupied by the gas before it was absorbed, and each of these spaces is covered on its endoplasm side by a cap of deeply stained oxidase widely open on the ectoplasm side. This cap is continuous with a ring of oxidase passing round the whole of the outer zone and connecting these caps with each other. The marginal zone when examined with a high power is found to contain no oxidase in the ectoplasm. The oxidase, therefore, lies on the surface of the endoplasm, and the gas-vacuoles are formed in the ectoplasm. This accounts for the arrangement of the oxidase as a socket open to the ectoplasm into which the gas-vacuole fits. The appearance of the indophenol specimens with gas-vacuoles confirms the account of their position given in connexion with the preserved specimen (fig. 7, Pl. 33).

In specimens killed with formaldehyde the indophenol reaction sometimes developed very slowly and in this case the indophenol blue first appeared in the marginal zone, more especially near the bases of marginal pseudopodia, that is to say, in the zone where oxidase activity is the first to appear in a stimulated Arcella. The stain gradually extended inwards from the marginal zone into the outer zone, and when staining was completed a faint blue appeared also in the apical area. The effect of the stain was not sharp, the whole of the cytoplasm had a pale blue tinge which did not extend into the nuclei. The deep stain seen round the gas-vacuoles and in the outer zone is due to the masses of large granules distributed in this part of the cytoplasm which takes on the indophenol blue in quantity. The distribution of these granules coincides roughly with the distribution of the chromidia, that is they form a ring confined to a narrow zone occupying the outer half of the outer zone of the cytoplasm.

Several attempts were made to stain Arcellae with indophenol without previous treatment with any reagent. In order to make the experiments otherwise similar to those just described the Arcellae were first reversed and then treated with the indophenol solution. In three such experiments no definite staining took place with the exception of a diffuse and very pale general blue tint uniform throughout the whole of the protoplasm. This result may be due, as will be discussed later, to the fact that the protoplasm of the living Arcella is disorganized and broken up by the amount of carbonate of soda in the indophenol solution. One Arcella when put into this soda solution was broken up by sudden exosmosis.

B. GUAIACUM.

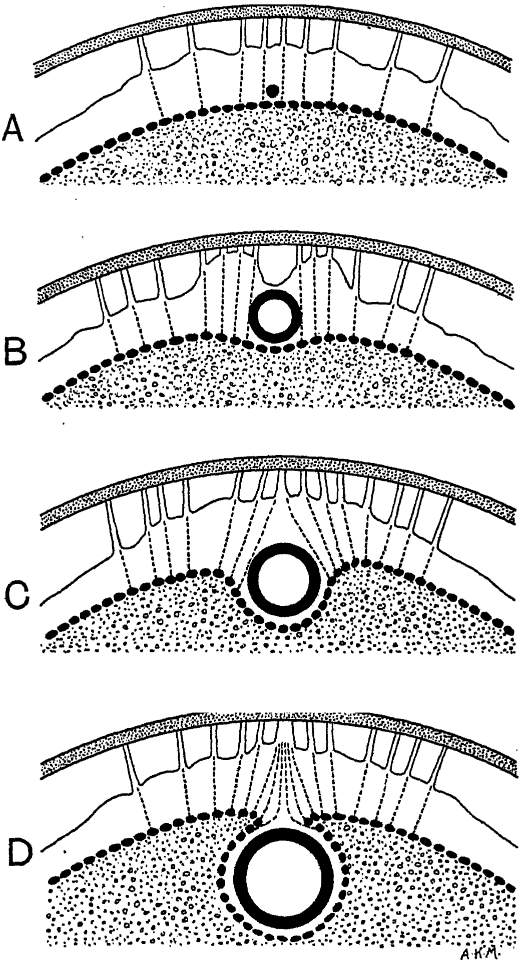
One or two experiments were made to test the effect of tincture of guaiacum on Arcella. In one experiment the Arcella was reversed and three gas-vacuoles were formed, one of them reaching a large size when the tincture was applied. The amount of alcohol in the tincture sufficed to preserve the protoplasm of the Arcella and the gas-vacuoles were observed in the dead specimen. In five to eight minutes the gas disappeared from the vacuoles, and as it went the cytoplasm became pale blue, of a deeper tint round the vacuoles and in the marginal pseudopodia. In fifteen to twenty minutes after applying the guaiacum a band of granules in the outer half of the outer zone of cytoplasm was quite distinct, occupying the same position as the granules which were most deeply stained in the indophenol experiment. In guaiacum tests the staining in this zone also commenced along the edge of the marginal zone and spread inwards.

C. BENZIDINE.

This reagent has been generally regarded as one of the most reliable of the tests for the presence of oxidases. It has even been used to discriminate between oxidase and peroxidase in the cell. All the results to be described were obtained without the use of peroxide of hydrogen. The solution of benzidine used was made up from equal parts of a saturated solution of benzidine in 50 per cent. alcohol, and a 0·25 solution of carbonate of soda in water. Equally good results were obtained in some cases with benzidine in 50 per cent. alcohol without the addition of the alkali. In these experiments only a very minute quantity of the benzidine solution was used, so that the 50 per cent. alcohol was diluted by the watery fluid containing the Arcella. It was found that undiluted 50 per cent. alcohol saturated with benzidine gave very faint or no colour reaction. The failure to stain may possibly be put down to the dehydrating action of the stronger alcohol stopping the enzyme action.

The first effects of the benzidine reaction in Arcella are seen in the marginal zone exactly as in indophenol and guaiacum stain. This consists in the appearance of a narrow band of granules stained blue along the outer circumference of the marginal zone just inside the ectoplasm. The stain then spreads inwards until it reaches the inner edge of the outer zone and it spreads inwards and upwards towards the obverse surface of the cytoplasm. The final result in an Arcella, where staining has been completed, is shown in fig. 9, Pl. 34. This Arcella, before it was killed, contained two large and one small vacuole at IX, VII, and V respectively, and the appearance of the Arcella when killed was extremely life-like, only a few of the anchoring marginal filopodia were retracted. The depth of stain varies in different parts of the cytoplasm. A slight diffuse stain is seen in the karyosomes and extends into the radiating processes, and one of the nuclei, at IV, has indications of radial rows of granules which appeared to be continuations of the spoke-like rays from the karyosome; these granules are stained more deeply than the scattered granules round them. As regards depth of colour there is no great difference in this respect in the areas occupied by the gas-vacuoles as there was in the case of the indophenol specimens. The only regular and invariable character in differentiation seen in all the benzidine specimens was the deep stain of the granules in the marginal zone as compared with those lying inside this zone. However long the staining is continued the latter never become as deeply stained as the former. A very marked feature in the deeply stained Arcella is the complete absence of any benzidine-blue granules in the central mass. As shown in fig. 9, Pl. 34, there is a sharply defined limit roughly parallel with the edge of the

TEXT-FIG. 1.



Schematic figure illustrating the screening action of the gas-vacuoles. For details see text, p. 596.

foramen, and lying immediately above it, in fact this limit coincides with the line along which the ectoplasm is attached to the inner obverse surface of the test. Along this line there is as shown in fig. 9, Pl. 34, a distinct row of small well-stained benzidine-blue granules. The same specimen showed very clearly the way in which the gas-vacuoles are placed in respect to the layer of oxidase granules, but it was not found practicable to indicate this clearly in the drawing without making it diagrammatic. A figure is therefore given in the text (Text-figure 1 D, p. 585) showing this point. When in the specimen the region of the gas-vacuoles is carefully examined by focussing up and down, it is found that a small patch on the outer surface of each large vacuole is not occupied by benzidine-blue granules. This gap is clearly seen in fig. 9, Pl. 34, as IX. The gas-vacuole here was large enough to fill the outer part of a sector of about 40°. In the hyaline protoplasm of the smaller internal lobopodia and of the internal filopodia there are no oxidase granules nor were there any in the large external pseudopodia. That is to say, the oxidase granules are confined to the surface of the endoplasm, and they only occur in the internal lobopodia when they become large and they are invaded by endoplasm. The oxidase granules, therefore, in the Arcella at rest, before gas-vacuoles are formed, are arranged in a ring round the marginal zone. This ring is continued as a layer of granules over the surface of the endoplasm upwards and inwards to the inner obverse surface of the test where the layer ends round the edge of the central mass where it is attached to the test.

D. PYROGALLOL.

This reagent was used because it was expected to give a more sharply defined limit to the distribution of the oxidase concerned in the production of gas. It was applied while the Arcella contained four gas-vacuoles and had firmly attached itself to the slide after righting. A strong solution of pyrogallol in water killed the Arcella instantaneously and the gas-vacuoles vanished in a few seconds. About one minute later a 3 per cent. potash solution was added, and in another minute the oxidase in the outer zone had turned dark brown with a sharp line exactly like that in fig. 9, Pl. 34, in which the row of benzidine-blue granules shows where the obverse protoplasm of the outer zone is in contact with the test. No structure is brought out in the protoplasm; where the oxidation of the pyrogallol takes place the colour forms a diffuse and uniform screen so that not even the nuclei are visible. The depth of stain is so intense after two or three minutes that, whether the nuclei are stained or not, they are invisible in the darkened cytoplasm.

E. ORTOL.

This reducing agent (the well-known photographic developer) is a mixture of orthomethylamidophenol and hydroquinone. It was tried because it reduces without the addition of alkali or in very weak alkaline solution.

An Arcella treated with a 1 per cent. solution of ortol in rain-water was killed instantaneously, so quickly that the marginal pseudopodia were not retracted. The staining effect produced was exactly like that of pyrogallol, but the staining process was very much slower and more gradual. The first part of the cytoplasm to be darkened was, as in the oxidase tests, the marginal zone, which turned light brown and the colour spread from this zone inwards, the first stained parts becoming darker as spreading progressed. At the end of an hour the stain had not reached the edge of the central mass, where the oxidase layer ends. Before the stain reached this point the experiment came to an end as the ortol had a curious effect on the protoplasm, gradually causing it to shrink. This shrinking was observed at the beginning of the experiment by its action on the marginal pseudopodia, these being retracted after death. It may probably be the result of dehydration by the phenol base of the reducer. The nuclei remained unstained for as long as the experiment continued.

F. PEROXIDE OF HYDROGEN OR CATALASE REACTION.

A crushing experiment was made (p. 556) on an Arcella with gas-vacuoles, one of the vacuoles which were pressed out, instead of being reabsorbed by the protoplasm, increased in size for nearly two hours until its diameter increased five and one-third times. A further crushing experiment was made in order to test the action of peroxide of hydrogen on a fragment of protoplasm containing a minute remnant of a gas-vacuole· After crushing, a piece of the protoplasm was found containing a gas-vacuole which was watched and seen to be shrinking. Eight minutes after crushing a weak solution of peroxide of hydrogen (about 1 per cent.) was run in and within thirty seconds the size of this gas-vacuole was measured. One minute later the diameter was six times greater and the vacuole continued to grow. The vacuole had increased in diameter about ninety times in forty-three minutes. This rate of growth corresponds to that described in the early experiment and indicates that the gas in both experiments may be the product of catalase action upon a peroxide formed by the oxidase activity of the cell. In the peroxide experiment there can be little or no doubt that the gas in the vacuole is due to the reduction of the peroxide of hydrogen, and the growth of the gas-vacuole in the peroxide experiment corresponds exactly with the spontaneous growth of the gas vacuole in the crushing experiment described above. The difference between the two cases is that the peroxide of hydrogen was supplied in one of them, and in the other the peroxide was present in the protoplasm.

CHAPTER VIII.—EFFECT OF LIGHT UPON THE ACTIVITY OF OXIDASE.

The object of this experiment was to find out whether the oxidase in a plant-cell which contained no chlorophyll was sensitive to light, and to determine the position of the oxidase, if present, in the cell by means of the leuco-methylene blue method.

A terminal branch of Elodea canadensis, about 2 in. long, was taken from an aquarium standing away from the window, and put into a solution of ordinary methylene-blue stain of 1 in 50,000, and remained there for twenty minutes. The sprig of Elodea was then washed in several changes of water, and a young leaf from near the tip was put on a slide in rain-water and covered with a cover-glass. The slide was then put on the stage of the microscope standing on the sill of a south window, so that an image of the sun could be reflected from the flat mirror on the object. The edge of the young leaf consists of a single row of colourless cells, and one of these in which the nucleus was visible was centred in the field of the microscope, and the Abbe condenser was focussed on it. When all the necessary adjustments had been made by diffused light, the Abbe condenser was racked down to avoid the focussing of heat rays on the cell, and after removing the eye from the eye-piece, the mirror was turned to throw the direct light from the sun on the object (zero time). After two minutes the mirror was turned to throw diffuse light on the object, and the cell was rapidly examined, to find that no obvious change had taken place. After an interval of about thirty seconds, direct sunlight was again turned on the cell for another two minutes, and then another examination was made, again without finding any change. At the next examination of the cell at the end of a further two minutes (seven minutes from zero), the following changes were observed: a single large plastid lying end on to the nucleus, and connected to it by a distinct fine thread, was the only part of the cell which was stained with methylene blue, and the stain extended into the thread.[1] The whole mass of the plastid was stained with methylene blue, and no other part of the cytoplasm or nucleus showed any trace of the stain. The presence of this stain in the plastid (which Guillermond considered to be of the same nature as the chondriosomes) shows that the plastid contains oxidase and substrate. A very striking change in the condition of the cell, observed as soon as observations were resumed at the end of the seventh minute after insolation had started, was the slow circulation of the cytoplasm, which must have commenced during the last two minutes, and practically simultaneously with the production of oxygen by the plastid. The circulation was beautifully clear, and was taking place in the typical manner round the somewhat irregular fluid vacuole.

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[1] I find that the threads connecting plastid and nucleus have been described by Lindström.

This experiment shows that in a cell which contained not a trace of chlorophyll, the oxidase is directly stimulated by light. The activity of the oxidase is accompanied by protoplasmic movements, and on the evidence furnished by the Arcella experiments, it is making no great assumption to attribute the movement to the influence of the oxidase activity on the surface tension of the interface protoplasm and vacuole-fluid.

CHAPTER IX.—DISCUSSION OF EXPERIMENTAL RESULTS.

A. The Nature of the Gas in the Vacuoles.

The original object of this investigation was to determine the nature of the contents of the gas-vacuoles in Arcella and the conclusions now reached on this point are, briefly stated, as follows :

(1) The gas in the vacuoles is pure oxygen.
(2) If any other gas is present, it is only in excessively minute traces.
(3) The oxygen is produced by oxidase action.
(4) The stimuli which bring about gas-vacuole formation can, so far as they are known at present, be classed under one head, however different they may appear at first sight ; they all stimulate oxidase action.

(1) The evidence that the gas in the vacuoles is pure oxygen is practically all circumstantial evidence, since it has not been collected and no direct chemical test has been applied. The nearest approach to such a test was made in the Engelmann experiment with Peranema, and this was not an application of the gas from the vacuole itself to the motionless Flagellate. The assumptions were made that the gas in the vacuole must be under pressure, that it was therefore in saturated solution in the protoplasm, and that it consequently would diffuse from the protoplasm into the water. Granting these assumptions the test gave as definite a result as any obtained by Engelmann. The escape of oxygen shown in this experiment to take place

from a holozoic protozoan is unique, and it seems perfectly safe to assume that the oxygen which is given off by the external pseudopodium must be derived either from the gas-vacuole or from oxygen which, instead of passing into the vacuole, went into solution in the protoplasm directly as soon as it was produced. The possibility that it might be given off in the ordinary respiratory gas-exchange may safely be excluded. An observation which shows that the external pseudopodia are permeated with oxygen is that described in the methylene blue experiments where a constant feature was the appearance of blue in the external pseudopodia before it showed elsewhere. The reason why the colour should first appear in this particular position will be discussed later, and it will be found that it is connected with the commencement of more intense oxidase action at the base of the marginal pseudopodia, and the consequent production of gas there.

The results obtained with the vital stains, taken together, indicate with a high degree of probability, if not with absolute certainty, that the gas produced is oxygen. Taken alone, the methylene-blue experiments are not demonstrative, for other reactions known to take place in the living cell might conceivably have caused the reduction and oxidation of the dye. However, these reactions which are known to give oxidation with methylene blue and toluidine blue would not give corresponding reactions with all the other vital stains used in these experiments.

The generally accepted view that the gas in the vacuoles is carbon dioxide, founded on the result of a single experiment by Bütschli, implies as already pointed out (p. 559) that the protoplasm of Arcella is immune to CO_2 poisoning. For in order to produce a bubble the pressure of the gas in the protoplasm must have risen to saturation point, at the least, and as the solubility of CO_2 in water is about 100, long before this point was reached the protoplasm would have been killed unless it differed from all other protoplasm. The experiments (p. 558) made with different percentages of dissolved CO_2 showed perfectly clearly that Arcella, if anything, is rather susceptible to CO_2 poisoning. Further, the experiments with caustic potash solution (p. 559) show not only that the gas in the vacuole is not CO_2 but that it must be one of the two gases forming the bulk of the air because saturation of the solution with air increased the time taken to dissolve the gas from the vacuole. This sequence of experiments points again to the gas being oxygen, as the inert gas nitrogen can be dismissed from consideration without further discussion.

The apparently paradoxical result that a decrease of oxygen partial pressure leads to the production of oxygen in Arcella is explained by considering the source of the oxygen. The gas production, it will at once be evident, is subject to the law of chemical equilibrium, which would indicate that the lower the pressure of one of the products of the enzyme activity, the greater will be this activity for the time being. Therefore these pressure experiments also go to prove that the gas produced, not only under experimental conditions, but also by the wild Arcella, where deficiency of oxygen or its total absence is of constant occurrence, is produced in direct response to this condition. The experiment with the methylene-blue Arcella under much reduced oxygen pressure shows that when this pressure becomes very low no gas-vacuole is formed, although the oxidase is active, as proved by the appearance of the oxidized methylene blue. Judging from all this, Arcella is semi-anaerobic and can live under reduced oxygen pressure until a certain point is reached, at this point, or just before it, gas-vacuoles are formed sufficient to carry the Arcella up to the surface of the water where the oxygen supply is unlimited.

The substantial piece of evidence which is almost conclusive that the vacuoles contain oxygen, is the presence in A. discoides of oxidase and its anatomical relations to the gas-vacuoles. The position of the gas-vacuoles is absolutely constant in this species, not only as regards the fully-formed vacuoles which are always in the outer zone of the protoplasm, but also as regards the commencing vacuole and the disappearing vacuole which are both invariably found in the marginal zone. This location of the gas-vacuoles in their different stages corresponds exactly to the arrangement of the oxidase. The first appearance of the gas-vacuoles always takes place where the tests with all the oxidase reagents show that the oxidase action is most powerful, namely, in the marginal zone. The fully-formed gas-vacuoles never extend into the central mass, they are confined, like the oxidase, to the outer zone, and when the gas-vacuoles disappear they always do so in the marginal zone.

(2) The question whether another gas is present is, I believe, answered by the contrasted behaviour of the disappearing vacuole in the standard experiment and in a methylene-blue experiment. If any other gas but oxygen were present in the vacuole, it would necessarily be a gas more chemically inert than oxygen. It would follow that after the absorption of the oxygen a residue of the inert gas would remain and persist for some time. As will be shown later the point at which the enhanced activity of the oxidase comes to an end can be determined by very clear indications in the behaviour of the protoplasm, and before this point is reached, the last traces of gas-vacuole have, without any exception, disappeared. The evidence from methylene-blue experiments is quite conclusive. The disappearing vacuole in these experiments alternately expanded as the methylene blue vanished in the adjacent protoplasm and shrank when the methylene blue reappeared. At the final disappearance of the gas-vacuole there is always an especially deep blue patch produced on that spot in the protoplasm, so that the last trace of gas in the vacuole is the same gas, viz. oxygen, as that which produced the alternate changes of colour during the dwindling of the gas-vacuole. Consequently, the probability that even a minute trace of any other gas is present is very remote.

(3) It is generally admitted that the identification and classification of oxidases is at present very uncertain. The classification which most satisfies the needs of the physiologist is that proposed by Bach. According to his methods of discrimination the oxidase found in Arcella is an oxidase in the narrow sense of the word, that is, not a peroxidase. All the oxidase tests

used gave the reaction without the addition of hydrogen peroxide. This result does not of course exclude the possibility of the existence of one or more peroxidases, and the experiment described on p. 587 shows the existence of a catalase in Arcella.

(4) It follows quite clearly from the experimental treatment of Arcella with reduced oxygen pressure that this reduction is an adequate stimulus for the first formation of gas-vacuoles. The production of the gas by an oxidase system shows why this stimulus should be efficient, but at first sight it does not in the least explain why gas-vacuoles should be formed in Engelmann's hanging-drop experiment (see p. 533) or in the standard experiment. The reversed Arcella in these experiments is certainly not suffering from any deficiency in the supply of oxygen as it is actually lying reversed either on the surface film of the hanging-drop or in a shallow-drop on a slide. While the pseudopodia are attempting to get a foothold on the surface-film of the hanging-drop a certain amount, which is, however, extremely minute, of oxygen is being set free in the protoplasm, as demonstrated by the early appearance of methylene blue in the external pseudopodia. A few minutes later the oxygen vacuoles appear, indicating that the oxidase has now reached a higher degree of activity without any apparent increase in any external stimulus to account for it. In fact, even if the initial stimulus which induces oxidase action is also in these experiments a reduction of oxygen pressure, that stimulus is no longer being applied, for even before the appearance of the vacuoles the protoplasm must, in the neighbourhood of the vacuole, be already saturated with oxygen. There must be some influence other than the original stimulus which continuously and increasingly acts upon the oxidase to enhance its action. This influence is, I believe, the manifestation of interaction between, on the one hand, the interface of the external pseudopodia and the water, and on the other hand the oxidase-substrate layer of the outer zone of protoplasm.

B. THE STIMULUS TO PSEUDOPODIUM FORMATION.

Pseudopodium formation and oxidase activity have been shown by the experiments on Arcella (pp. 583, 586) to be closely related. Believers in Verworn's biogen hypothesis will have no difficulty in explaining the movements of the protoplasm by the stimulating action of oxygen on the biogens, as Verworn himself has done (M. Verworn, 1909). If we had any knowledge of what a biogen really is and of the way it behaves in the presence or absence of oxygen, this might be an explanation, but without this knowledge our insight into the functioning of protoplasmic movement is not advanced by this hypothesis.

The problem to be solved in the case of Arcella is whether the protoplasmic movement is due indirectly to the stimulation of an increased supply of oxygen, or to the direct influence of the oxidase activity on the protoplasm. The difficulty in deciding between these two alternatives lies in the circumstance that in point of time it is impossible to separate them. When the oxidase activity is stimulated the oxygen production is increased and simultaneously active protoplasmic movements are started ; oxidase activity, oxygen production, and protoplasmic movement, reach their highest manifestation together, and together they subside until they come to rest. As no clue is to be obtained from this consideration, a different way of attacking the problem must be taken. This consists in the analysis of the interaction of these factors in the different parts of the organism. The experiments on Arcella show that: (1) Marginal pseudopodia are constantly formed where no gas-vacuole appears at all, that is marginal pseudopodia are formed without the most intensive oxygen production which forms gas-vacuoles; (2) marginal pseudopodia are not always formed where the vital stain experiments indicate a high degree of saturation with oxygen. These two results prove, I believe, that there is no immediate connexion between the amount of oxygen in the cytoplasm and pseudopodium formation.

Pseudopodial activity is not brought about by saturation of the protoplasm with oxygen, otherwise the whole circumference of the marginal zone would be covered with active lobopodia and filopodia. Actually the outburst of pseudopodia only takes place at six or seven points at the most, and in each of these outbursts, when a gas-vacuole appears there is a spot where at a certain stage pseudopodia are absent. This stage is shown in fig. 2 D, Pl. 30, and has already been described as the crater or notch-stage in the outburst of marginal pseudopodia.

C. THE SCREENING ACTION OF THE GAS-VACUOLE.

The figures A–D in Text-figure 1 p. 585 illustrate in a diagrammatic fashion the relations of the parts round about an expanding gas-vacuole. From above downwards in each figure is the test, the marginal space with filopodia crossing it, the ectoplasm, the gas-vacuole, the layer of oxidase-substrate granules, and the endoplasm.

In these diagrams the results of such observations as have been pictured in fig. 2, Pl. 30, fig. 5 A–J, Pl. 32, and fig. 9, Pl. 34 (benzidine reaction) are combined. They show that the oxidase-substrate lies in a single layer of granules on the surface of the endoplasm and that the oxygen is secreted as a gas on the outer surface of this layer into the ectoplasm. As the gas-vacuole expands it pushes the layer of oxidase-granules inwards more and more (Text-figure 1 B and C) until finally an invagination of this layer is formed with a constricted opening (D) towards the ectoplasm. For the sake of diagrammatic clearness the gas-vacuole is not drawn in close contact with the oxidase layer, they touch in reality. The meaning of the dotted lines crossing the ectoplasm will be explained later (p. 597). All that is necessary to say now is that the lines indicate the direction of the path of a radiation from the oxidase-substrate granules which impinges on the portion of the surface-film of the ectoplasm lying opposite each granule.

The discussion is now concerned with Text-figure 1 B. In this diagram the gas-vacuole and the adjacent structures are shown at the stage drawn from life in fig. 2 D, Pl. 30. It illustrates the formation of the notch or crater in the ectoplasm just outside the rapidly expanding gas-vacuole. When this feature in the movements of the ectoplasm had once been recognized it was found invariably to accompany this particular phase of vacuole expansion. What happens in the ectoplasm is that an area of surface-film, about equal to an equatorial section of the gas-vacuole, loses its filopodia and suddenly sinks below the level of the broad lobopod, until it almost meets the gas-bubble. By the time, only about one minute later, when the bubble has expanded to the extent shown in Text-figure 1 c, the depression in the ectoplasm surface-film has already disappeared, the formation of a notch or crater being always quite a transitory feature. The sudden rush of the surface-film down to the gas-vacuole is very striking, and gives, as I interpret it, a demonstration of the effect produced by a rapid increase of surface-tension in the surface-film of the ectoplasm. As the dotted stream lines in diagram 1 B indicate, the crater area, when this particular size is reached by the gas-bubble, is shielded from the radiation emanating from the oxidase-granules, which elsewhere is acting on the surface-tension, reducing it and thus causing protrusion of pseudopodia. When the size of the oxygen bubble has increased proportionately to that shown in Text-figure 1 c, the bubble has buckled the layer of oxidase-substrate granules so much that the radiations from them have, all round the bubble, a free course to the surface-film, and are no longer intercepted by the bubble with the result that the surface-tension of the ectoplasm is again reduced in the crater area, and pseudopodia are again protruded from the spot where the notch existed.

These observations, I believe, decide the question whether the activity of the marginal pseudopodia is due to the presence of oxygen, or whether it is connected with the activity of the oxygen-substrate granules. The screening action of the oxygen bubble shows conclusively that the activity of the oxidase-substrate layer must be concerned in the formation of the marginal pseudopodia and filopodia.

Further, it seems clear that changes of surface-tension play a part in the protrusion and retraction of pseudopodia.

PART II.

THE INTERFACES OF THE CELL AND THE CYTOPHRAGM HYPOTHESIS

CHAPTER X.—THE CYTOPHRAGM HYPOTHESIS.

THE two structural elements which are mainly concerned in the formation of marginal pseudopodia in Arcella are (1) the interface protoplasm water, and (2) the oxidase-substrate layer. These two elements are normally separated by a small but appreciable distance, the thickness of the ectoplasm, and it is therefore necessary to account for the action of one upon the other by some connecting agency. For this purpose a working hypothesis is suggested which will be called the ' Cytophragm hypothesis '.

This hypothesis involves the use of some new technical terms. What has hitherto been known as the 'cell-membrane ' I propose to call ' ectophragm '. A number of objections can be raised to the use of the term ' cell-membrane ' in biology. In the first place this expression implies the presence of a physical membrane on the surface of all cells, whether its presence can be demonstrated under the microscope or not. When dealing with what is called naked protoplasm, such a membrane, has not been shown to exist. Moreover if the interface protoplasm water has certain properties in common with what are known as semi-permeable membranes, it has not yet been shown that such an interface cannot have these properties without the existence of an actual solid membrane. In the second place a very large number of animal and vegetable cells are bounded by a visible envelope, very often membraneous, which may or may not act as a semi-permeable membrane, but is a cell-membrane.

Where the surface activity of protoplasm actually comes into play during physiological processes the interface of the living protoplasm and the surrounding medium is the seat of the changes both chemical and physical which play sometimes a secondary part, but frequently a chief part in functional processes. If an actual physical membrane is supposed to exist on such a surface, the extremely minute and delicate changes at the surface can only occur if such a membrane is entirely different from anything that can be produced physically, or from anything that has been observed on a protoplasmic surface. In fact it has often been pointed out that the actual thickness of a ‘ cell-membrane ’ cannot be more than that of a single molecule. A membrane of this nature would not differ from the ‘ limiting membrane ’ of, say, a drop of water resting on a solid surface in air. In this instance the so-called limiting membrane consists of water molecules. In the case of the ‘ limiting membrane ’ or interface of protoplasm in water, the protoplasmic surface contains elements derived from the protoplasm ; but its composition cannot, owing to the action of the second law of thermo-dynamics, contain these elements in the same proportion as the internal mass of the protoplasm.

Among the constituents of protoplasm which tend to accumulate in the ectophragm are the potassium-ions which Macallum (1905, 1911) has demonstrated to occur there in a large number of functionally active cells. He attributes the concentration of the K-ions to the action of the Gibbs-Thompson law. As these electropositive ions must polarize the surface-film of the protoplasm, it follows that only a limited number of such ions can accumulate, as the polarization itself would check the entrance of more than a limited number of such ions into the surface-film ; and this would check the action of the Gibbs-Thomson law. In the cytophragm hypothesis the main thesis is that there is a driving force which acts in forcing a greater charge of K-ions into the ectophragm than would enter it under the conditions contemplated by Macallum. In Arcella this driving force is according to my working hypothesis the polarization of the oxidase-substrate granules. There is thus produced an internal polarized layer parallel to the external surface of the protoplasm of the outer zone. Such an internal polarized layer imbedded in the protoplasm influencing the cytophragm I propose to call an ‘endophragm ’. The one assumption made in attributing a polarized condition to the endophragm is that the substrate is in a colloidal condition. There is at present no actual proof that the substrate is in this state, but from its reaction to the benzidine test it is clear that the substrate, which is the bulky part of each oxidase substrate granule, has a high hydrogen-ion concentration. The oxidase portion of each granule may safely be taken as being colloidal like the majority of enzymes, and, according to Wolff's experiments, the surface charge of this colloid is electronegative, as he demonstrated that the oxidase action reaches an optimum, and can only take place in an alkaline medium. If this view of the opposite charges of the enzyme and its substrate is correct, it would account for the behaviour of such enzyme complexes as will be pointed out later. In the particular instance of the endophragm of Arcella, the charge of the substrate would bring about the production of an electropositive layer which is, however, not a continuous one, as the oxidase-substrate granules always remain discrete.

Considering the action of a single oxidase-substrate granule on the cytophragm hypothesis the rapidly increasing activity would result in a rapid rise of the positive charge on the substrate, and this would lead to the formation of a stream of positive ions from this surface. Following for the moment that part of the stream which passes outwards into the ectoplasm, and taking the velocity of the ions in protoplasm as proportional if not equal to the velocity in water, the leading ion will be the hydrogen ion. Following the H-ions there will be a ' procession ' of ions following the order potassium, sodium, calcium, the latter two being far behind the potassium ions. Whether the sodium and calcium ions reach the surface or not depends on the state of polarization of the cytophragm which has been produced in advance by the potassium ions.

Before discussing the action of the kations on the cytophragm, it must be mentioned that an essential part of the cytophragm is the layer of anions on the outer side of the interface. The electrostatic double layer is not, I consider, a true Helmholtz double layer, as there is not for any appreciable time a balance between the electropositive and the electronegative charges in the functioning cell. The accumulation of the anions in the outer layer, sufficient to balance a sudden accumulation of K-ions in the inner layer, is a question of time ; and seeing that although the supply of OH-ions in the surrounding medium is unlimited, their concentration is low. On this view the supply of OH-ions will follow the law of mass action. The result of this will be that the conditions in the cytophragm will be of an alternating character, and resemble the conditions of the surface film of the mercury in Bredig's well-known experiment of the globule of mercury immersed in a solution of mercuric iodide. For the sake of clearness, only that part of the cytophragm which lies on the outer surface of the cell, I propose to call ectophragm.

In the Protozoon itself the cytophragm is not entirely on the external surface. When food is ingested, the lining of the food-vacuole is composed of a detached portion of the ectophragm which wanders into the interior of the cell, but remains in spite of this an external surface. In the same way the lining of any fluid-vacuole or gas-vacuole lying in the protoplasm is an external surface, which can be considered as a part of the cytophragm.

It is now possible to give the definition of the new terms which have been introduced. The cytophragm in Arcella is the interface which has protoplasm on the one side, and on the other side either (1) surrounding water, or (2) a fluid which is not a part of the dispersoid system of the protoplasm. Where the cytophragm is in contact with surrounding water, the electrostatic charges are positive internally and negative externally, and this portion of the cytophragm is the ectophragm. The endophragm in Arcella may consist of something more than the oxidase system which lies below the free surface of the outer zone protoplasm ; but at present this is the only part of the endophragm which is definitely located. The endophragmal system may be defined as the sum of the polarized surfaces of the colloidal phase in the protoplasm which acts upon and reacts to the polarized ectophragm. A discussion of the reaction of the endophragm to the ectophragm is postponed until the end of the next chapter.

CHAPTER XI.—THE INTERREACTION BETWEEN ECTO- AND ENDOPHRAGM.

A. ELECTROLYTIC HYPOTHESIS OF OXIDASE ACTION.

TAKING the interaction of endophragm and ectophragm in Arcella as established, it must now be discussed how it is brought about. I believe that the clue to this interaction is given by Macallum's experiments on the distribution of the potassium ions in animal and vegetable cells and the changes in surface tension which he attributes to the wandering of the ions into the ectophragm in consequence of the application of the Gibbs law. Macallum's observations, together with Wolff's results from experiments on the influence of increased alkalinity on raising the intensity of oxidase action, may be used to show how it may be possible that changes in the activity of the oxidase can cause protoplasmic movement.

The oxidase in Arcella lies in a continuous layer formed of discrete granules on the surface bounding the ectoplasm internally, and lying on the endoplasm. The layer of oxidase extends parallel to the surface of the protoplasm which is exposed to the fluid in the marginal space. The oxidase is a system of enzyme and substrate, so that a portion at least of the substrate lies between enzyme and ectophragm. When oxidation takes place the electrical charge of the substrate is increased as there is 'so far as ions and their derivatives are concerned a gain of positive charges by the substance to be oxidized' (Ostwald, 'Outlines of General Chemistry', 1912, p. 401). Applying this principle to the oxidase granules the result would be that the substrate surrounding each granule would receive a positive charge while the oxidase itself would have a negative charge. Each of these systems will turn towards the ectophragm a side charged positively, and a stage of potential will be reached at which the kations in the solution will be discharged towards the ectophragm. They will travel through the ectoplasm at different rates of speed hydrogen ions first, potassium ions second, and so on. Neglecting for the moment the H-ions, we find that this behaviour of K-ions agrees with the experimental results of Macallum and others. The ectophragm being impermeable to potassium ions they will collect there and polarize it, so that if a Helmholtz double layer does not already exist it will now be formed on the ectophragm. Should the ectophragm be permeable to H-ions their arrival at the ectophragm will discharge the Helmholtz double layer, and this would give, if a record could be made of the electrical tension of the ectophragm, a negative variation, as the first indication of oxidase action and would be followed immediately by a strong positive deviation. This view expresses the electrolytic part of a rough working model of oxidase and the surface of the cell, and if the hypothesis on which it is based is sound it should help to throw light on phenomena apparently quite unconnected with the action of oxidase in Arcella.

Macallum's conclusions on the way in which the ions reach the surface-film are supplemented by this view of the influence exerted on the ectophragm by the layer of oxidase in Arcella. The kations which pass into the ectophragm do not enter it entirely as a consequence of the action of Gibb's law, but are projected into it from the polarized oxidase system. The result is the same whether the electrolytic, the thermo-dynamic, or both methods are at work.

The application of this electrolytic hypothesis of oxidase action to an explanation of gas-vacuole formation in Arcella can only be used if it can be shown that the interaction of oxidase and ectophragm is reciprocal, in other words that the polarization of one determines the polarization of the other.

It has already been pointed out that reduction of oxygen pressure stimulates oxidase action, but in the case of Engelmann's drop experiment the reversed Arcella formed gas-vacuoles without there being any lack of oxygen. In the standard reversal experiment there is no need to postulate a reduction of oxygen pressure, as the conditions are sufficiently similar to those of the Engelmann experiment. They can be grouped together in explaining the remarkable result of gas-vacuole formation as a consequence of reversal.

When Arcella is reversed the great change made in its external relations is that the external pseudopodia which were in contact with a solid substratum are now only in contact with water. This change must necessarily bring about a change of polarization in the ectophragm of the external pseudopodia, and as the water is alkaline the change is one which involves a condensation of anions (hydroxyl ions) on it. This condensation will necessarily be very slight, but, however slight, it will act on the polarization of the oxidase layer, and this again will act on the ectophragm by a condensation of kations. The reduction of surface tension results in the formation of external pseudopodia, which not being able to reach a solid surface become much extended and thus greatly increase the area of the ectophragm with its double Helmholtz layer. It is the influence of this large polarized surface which on this view stimulates the oxidase system to the height of activity sufficient to produce a peroxide which split by catalase forms the gas-vacuoles.

Experiments with the reduced partial pressure of oxygen clearly demonstrate that the oxidase can be stimulated by want of oxygen, but the stimulation, it is equally clear, ceases as soon as the molecular oxygen appears. In the reversal experiments which are now being discussed the oxidase begins to show its activity as soon as the first external pseudopodium is protruded, for the methylene blue reappears at this stage. The oxygen being now supplied, the subsequent activity of the external pseudopodia, however great it may be, does not cause lack of oxygen to affect the oxidase since the oxygen gas-vacuoles are formed.

The inference to be drawn from the reversal experiments regarding the stimulation of the oxidase and the formation of gas-vacuoles is that it must be attributed to (1) interaction between ectophragm and oxidase layer (endophragm), and (2) increase of alkalinity in the ectoplasm which stimulates the activity of the oxidase system, which in producing oxygen brings about the formation of gas-vacuoles.

B. Action of Oxidase upon Formation of Pseudopodia in Arcella.

The experiments described on pp. 541–64, and illustrated in figs. 1 and 2, Pl. 30, fig. 5, Pl. 32, and fig. 10, Pl. 35, show how marginal pseudopodia are produced as a result of oxidase activity. This result is of far-reaching importance for the understanding of protoplasmic movement. The hypothesis formulated is to account for the energy set free by the oxidations and reductions (brought about by the catalytic action of the oxidases) being converted into the energy used in pseudopodial movement. In order to do this it must first be shown that there is an intimate connexion between the activity of the oxidase layer and changes in the surface tension of the ectophragm. This part of the subject has already been dealt with above, and it now remains to be seen in what way the stream of ions poured on the ectophragm from the functioning oxidase can cause the sudden outflow or outburst of the lobopodia and filopodia. The view adopted in accounting for such movements is that they are primarily brought about by an alteration of the surface tension.

As Macallum pointed out, the concentration of ions in the surface-film while increasing the electrical tension, at the same time lowers the surface tension. In addition to this physical action there must also be chemical changes taking place as a result of the accumulation of ions in the ectophragm. This accumulation must cause changes of chemical equilibrium which will follow van't Hoff's law. The first effective ions to reach the cytophragm are the potassium-ions, and these, as Macallum's tests indicate, are present in quantity. As a part of the equilibrium to be established there is the formation of potassium hydrate from the potassium-ions of the inner side of the Helmholtz double layer and the hydroxyl-ions of the outer layer. The potassium hydrate thus formed will combine with the triglycerids, lipoids, &c., which according to the Gibbs-Thomson law are present in the ectophragm. The resulting saponification of the fats will still further reduce the surface tension of the ectophragm however slight the total amount of hydrolized fat may be. There are thus at least two agents, one physical and one chemical, at work, both tending to reduce the surface tension of the ectophragm lying opposite to active areas in the oxidase layer, and these two agents combined are clearly sufficient to account for pseudopodial formation by reduction of surface tension. The reason why in some cases a lobopod and in others a filopod is formed can now quite simply be explained; a lobopod is protruded when the oxidase activity is uniform over an area corresponding roughly to the area of the base of the lobopod, while a filopod will be shot out when a sudden increase of activity takes place in a single oxidase granule or small group of granules. This way of accounting for the formation of pseudopodia explains the great difference in the rapidity of movement between a slowly extended lobopod and a rapidly projected filopod. The lobopod is extended when a correspondingly large area of the oxidase layer gradually becomes active as the result of a stimulus, the first reaction of the oxidase being weak and becoming gradually more intense. The filopod is formed from the surface of the lobopod by a sudden increase of ion production from a minute area in the oxidase layer.

When a lobopod is formed in Arcella the extended protoplasm consists of ectoplasm only, the outline of the endoplasm remaining unaltered. The quiescent protoplasm is covered by a layer of ectoplasm so thin that it is hardly distinguishable except at the bases of the anchoring filopodia. It follows from this that the growth of a marginal lobopod cannot be due to an accumulation of existing ectoplasm. The ectoplasm in the lobopod, therefore, must consist of modified endoplasm in which the granules have disappeared from view. As the endoplasm passes out into the lobopod it traverses the layer of oxidase where hydrolysis is in operation, and the minute granules which filter through being of a lipoid or fatty nature are hydrolized and become perfectly transparent. On this view the ectoplasm of a lobopod is composed partly of the original ectoplasm and partly of the converted endoplasm. The conversion of granular protoplasm into hyaline protoplasm is carried out when an opaque ovum containing finely divided yolk-granules is made transparent by the action of an alkali which hydrolizes the yolk-granules.

If this view is the true one it should explain the difference between the rhizopods with reticulose pseudopodia and those with lobose pseudopodia as regards the behaviour of the pseudopodia. The reticulate nature of the pseudopodia in Polystomella is the result of lateral branches being formed from filopodia: these branches on the oxidase hypothesis of pseudopodium formation must have at their base an oxidase granule; if this is so the benzidine or some such colour test should demonstrate its presence. Accordingly the difference between reticulose and lobose pseudopodia consists in a difference in the distribution of the oxidase and in the latter group it is confined to the base of the lobopod, whereas in the former the endoplasm penetrates into the finest filopodia, carrying with it the granules of oxidase.

CHAPTER XII.—FLAGELLA, CILIA, MITOTIC SPINDLE, AND CENTROSOME.

At the end of the last chapter the conclusion was reached that the movements of the pseudopodia from their protrusion to their disappearance is connected directly with the activity of the benzidine oxidase, which must influence the activity of the cytophragm.

If the morphological correspondence between the pseudopodia on the one hand and flagella and cilia on the other may be accepted as established, the source of motive power in all three of these organs must be the same. The instances which have been described in which a pseudopodium is gradually transformed into a flagellum as in Mastigophora, &c., demonstrates this completely. It is therefore to be expected that the same, or a similar oxidase which was found in Arcella, would also be found in close connexion with the flagella or cilia in other Protozoa.

When the benzidine test was applied to several Flagellates and Ciliates under conditions similar to those which gave the positive results with Arcella, it was found that the benzidine oxidase was actually present in all the various Protozoa examined.

A. EXPERIMENTS ON FLAGELLATES.

The first organism experimented on was Chlamydomonas. The insertion of the two flagella is in a clear part of the protoplasm free from chlorophyll, and the result of the benzidine test in this part of the cell is perfectly distinct. The insertion of each flagellum on either side of the pointed end is marked by a minute spot of the characteristic benzidine blue, the spot being just within the outline of the rostrum, not in the base of the flagellum. That is to say it bears the same relation to the flagellum as the basal body which has been described in other Flagellates bears to the flagellum or similar organ in them.

In Polytoma uvella treated with benzidine the basal body at the insertion of each flagellum stood out clearly as a minute blue spot.

The next Flagellate tested was Euglena viridis. The flagellar apparatus in Euglena teres has been described in detail by Wager (1900). The base of the flagellum indicates that the single lash is really composed of two fused flagella, for the insertion is bifurcate. The infundibulum in Euglena viridis has a long cylindrical mouth, and tracing the flagellum from the outside inwards it is found to be swollen at the inner end of the passage; beyond the swelling it is still a single filament and does not divide until the centre of the spherical inner portion of the funnel. At this point the flagellum divides into two filaments, one of which is in a line with the external part of the flagellum; the other makes with this an angle of about thirty degrees. This arrangement is constant, and the two points at which the flagellum is inserted have therefore a very definite position on the infundibulum. After killing the Euglena with 4 per cent. formaldehyde solution, and before applying the benzidine, the flagellum was, especially inside the infundibulum, difficult and sometimes impossible to trace, and the fact that the double insertion of the flagellum was known to be constant helped to show that the test in every case gave the same result.

The infundibular region is in Euglena viridis free from chlorophyll and quite hyaline. The benzidine blue does not appear in this portion of the protoplasm except in four positions, one at the base of each root of the flagellum, one in the swelling on the flagellum and one on the surface of the ' eyespot '. The stigma, as in Euglena teres, is exactly opposite the swelling on the flagellum. The two spots at the insertion of the flagellum occupy the position of two basal bodies, and are in bulk equal to the basal bodies shown in Wager's figures as stained by the iron-haematoxylin method. The spot in the swelling on the flagellum brought out by benzidine has likewise the same bulk as the dark body shown in Wager's figures in a similar position. The blue spot brought out by the benzidine in the stigma seems to lie on the surface of the pigment on the side turned away from the infundibulum.

It is interesting to note the close association of benzidine oxidase with the pigment spot.

In both Chlamydomonas and Euglena the chloroplast contains scattered patches of benzidine oxidase which stain more or less deeply in different parts. The more deeply stained patches seem to lie on the pyrenoid bodies. I could trace no connecting strands between the anterior basal bodies' oxidase in the hyaline protoplasm and those in the chloroplast.

B. Experiments on Ciliates.

Using the same methods of killing and of staining with benzidine, the following results were obtained on Paramecium and Coleps as representatives of the Ciliata. The results described were all obtained from specimens killed with concentrated corrosive sublimate solution, as it was found after careful comparison that the condition after killing with this or with formalin (1 in 10) was exactly the same for the structures which had to be examined. The advantage of using sublimate was that the cilia were well preserved and distinct after using this reagent. In no instance was there any difficulty in getting the benzidine reaction; this is interesting, because many, if not all, chemists who have worked on oxidases state that the enzyme action is checked by sublimate as completely as it is, for instance, by cyanides. That this 'poisoning' does not take place may be due partly to the fact that we are dealing here not with an unorganized mass of oxidase, but with an organized system of oxidase and cytophragm. Of course the action of the sublimate solution was made as short as possible, and often did not last longer, at any rate in its concentrated condition, than a fraction of a minute.

(*a*) Paramecium aurelia.

The action of the benzidine is rapid and its full effect is seen in twenty minutes to half an hour. A low-power view of the result on Paramecium aurelia is shown on fig. 11, Pl. 36. The whole surface is covered with closely set small blue spots, which have no very obvious arrangement but correspond apparently in number and position to the distribution of the cilia. Internally the two micronuclei have taken a deep stain and the macronucleus stands out from the rest of the protoplasm very clearly, the whole mass being stained pale grass-green and the surface of it covered with closely set spots of oxidase.

Under a high power the whole of the endoplasm is seen to be filled with a reticulum of oxidase filaments continuous with a similar reticulum permeating the substance of the macronucleus.

The surface of the Paramecium under an oil-immersion objective shows the appearance drawn in fig. 13 A, Pl. 36. The long axis of this drawing is parallel with the axis of the infusorian and the reticulum, consisting of pale blue strands with deep blue nodes, is seen to be roughly arranged in relation to the long axis. The arrangement is on the whole a system of parallel longitudinal strands with nodes at fairly regular intervals and cross strands connecting the laterally adjacent nodes. The deep spots at each node are found by careful focussing to lie each at the base of a cilium in the position of a basal body. The connexion between cilium and basal body is seen more clearly in fig. 13 c, Pl. 36. The oxidase strands which run longitudinally are the more regular owing to the arrangement of the basal bodies in longitudinal rows; the transverse connexions run at any angle to the long axis. This patch (fig. 13 A, Pl. 36) is situated at the hinder end of the body in the position shown in fig. 13, Pl. 36, marked *A*.

The patch marked *B* in the outline fig. 13, Pl. 36, is shown in detail in fig. 13 B, Pl. 36. This area lies across the edge of the peristome as indicated in fig. 13, Pl. 36. The edge of the peristome is clearly defined by a sudden change in the arrangement of the basal bodies; inside the peristome the arrangement is less regular (in the upper part of the figure); outside the peristome the basal bodies are arranged with great regularity as the nodes of a rectangular network spread on a curved surface, so that the basal bodies and the strands of oxidase connecting them form series of curves intersecting at fairly regular intervals. The arrangement of the basal bodies inside the peristome is less close-set than it is on the general surface, and fig. 13 B, Pl. 36, brings out very clearly a distinction between the peristomial network and the general surface network.

The patch shown in fig. 13 c, Pl. 36, situated at the anterior end of the Paramecium, showed more clearly than elsewhere how each basal body is connected with a cilium, and that no cilium is without a basal body. Here again the longitudinal lines of basal bodies, although not quite straight and regular, are quite evident. Very careful examination of the cilia failed to show that they contain any oxidase; the faint blue tinge of the cilia could be sufficiently explained by reflection from the curved surface of the cilia of the scattered blue light from the stained parts of the body.

There are no parts of the surface in Paramecium without cilia, so that it was not possible to find out whether the basal bodies may occur without bearing cilia. No basal bodies were found which were not connected by strands of oxidase with adjacent basal bodies, so that the whole surface of Paramecium is covered with a close network of oxidase strands covering the entire surface.

The new fact brought out in the above description is that the basal bodies and the connecting strands, first described by Schuberg, are composed of or contain a large amount of benzidine oxidase. This fact, when considered side by side with the relation between oxidase action and protoplasmic movement in Arcella, points to the probability that the same underlying principle accounts for the motility in both cases. In these two, as well as in the case of the flagella, the co-ordination of oxidase action and cytophragm action allows the energy generated by the enzyme to be converted into the energy which is dissipated by the movements of pseudopodium, flagellum, or cilium as the case may be. How the co-ordination is brought about is a question which must be considered with what is known about galvanotropism, thigmotropism, and chemotaxis in Protozoa.

(*b*) Coleps hirtus.

This armoured infusorian has a reduced covering of cilia, a condition obviously related to the acquirement of armour plates. The arrangement of the cilia is definite and constant, and has been carefully described by Maupas.

In fig. 14, Pl. 37, the arrangement of the cilia is shown in a specimen killed with sublimate. The cilia are much longer and stouter than they are in Paramecium, and the basal bodies are larger in proportion. They are stained deep blue by the benzidine method, and are connected by a similar network just below the ectoplasm as they were in Paramecium. It is interesting to note the arrangement of this network, the meshes of which are actually of similar size to the network of Paramecium so that the connexion between each basal body and the next is established through the thread joining five or six intermediate nodes in a somewhat zigzag line. At each angle of the network there is a distinct thickening of the oxidase much smaller than the basal body and smaller also than the basal bodies in Paramecium. These can be safely regarded as the vestigial basal bodies of an ancestral close-set covering of cilia with the connecting strands still persisting. These strands still serve as a connexion between the functional basal bodies. The entire surface is covered by this network without any change in character in any region of the body. As in Paramecium, a similar network pervades the whole of the endoplasm, and is continued and connected with an oxidase reticulum in the macronucleus and micronucleus. This endoplasmic network is also continuous with the superficial network connecting the basal bodies and lying just internal to the ectoplasm. The character of the network inside the macronucleus and inside the micronucleus[1] is in each case different from the endoplasmic network, and the oxidase takes on a different stain in the nucleus, being here more diffuse and paler, while the nodal thickenings are larger, more diffuse, and very close together. The network in the micronucleus is very sharply defined, the nodes are as deeply stained as the basal bodies, and the distance between them is about the same as the distance between the nodes in the superficial network. The connecting strands in the micronucleus are also well stained and sharply defined, if anything, rather finer than those in the superficial network. The appearance of the reticulum in the micronucleus has accordingly quite a character of its own. The various points described above are all clearly brought out in fig. 14, Pl. 37.

C. EXPERIMENTS ON NEPHRIDIUM CELLS OF LUMBRICUS.

In order to decide whether the basal bodies in ciliated cells reacted to benzidine a nephridium of an earthworm, Lumbricus herculeus, was fixed with sublimate and treated with benzidine solution in exactly the same way as the Protozoa.

The best cells for observation are those at the edge of the nephrostome. One of these is shown in optical section in fig. 15, Pl. 38. The whole of the cell is filled with a close reticulum stained with a blue of a similar tint to the network in the ciliates. As seen in optical section the free surface of the cell was apparently not covered with any superficial layer or 'Saum', but the basal bodies seem to lie actually in the surface layer as shown in the figure. The basal bodies do not appear to be absolutely uniform in size as stained by this method, but the small differences seen may be nothing more than an expression of different states of activity in the oxidase. The attachment of each cilium to a basal body comes out with absolutely diagrammatic clearness in these cells. In optical section the strands of oxidase which connect the basal bodies are not very distinct, but they are quite obvious when the basal bodies are focussed on a free surface of one of these cells lying in the plane of focus. As fig. 15, Pl. 38, shows, the long slender cilia have a blue tinge, but it is very difficult to decide whether this is due to actual benzidine staining or to reflection of blue light from the great mass of stained cells lying below the cell under observation and out of focus. If the stain were due to oxidase it might be expected that the distribution in the cilia would be uniform whereas as fig. 15, Pl. 38, shows, the basal part of the cilia is coloured in a way which suggests that the extent of the deeper blue depends on the amount of curvature. The cilia on the left, which are more sharply bent over near the base, are only coloured deeply as far as the bend, while the right-hand slightly curved cilia are deeply coloured farther out from the cell.

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[1] In fig. 14, Pl. 37, the micronucleus of Coleps is drawn for the first time, as Maupas' methods did not stain it. The body called nucleolus in his figures is, as Yves Delage suspected, not the micronucleus ('Traité de Zool. Concr.', tome i, p. 438).

In fig. 15, Pl. 38, the optical section was taken through the equatorial plane of the nucleus and showed the relation of the cytoplasmic oxidase network and the nuclear network. The network is continuous throughout, the meshes in the nucleus are slightly more open, but otherwise there is no difference, which is a striking fact. The nucleolus is distinct and contains a karyosome which is stained blue, but the oxidase network of the nucleus is continued without a break and without modification through the nucleolus.

In one preparation the pressure of the cover-glass had slightly separated two of the nephrostomial cells, and in the narrow chink between the two, strands of oxidase stained blue could be seen connecting the oxidase networks of the two cells.

It was very striking to observe in the nephridium that only those cells which bore either cilia or a flagellum contained any of the benzidine oxidase; all other cells were perfectly transparent and unstained.

D. Experiments on Mammalian Gonocytes.

The resemblance between the association of basal body and cilium on the one hand and centrosome and some motile organ on the other hand, has been familiar since the centrosome was described by Boveri as 'the dynamic centre of the cell'. The successful staining of the basal bodies in so many varied kinds of cells suggested that the same result might follow the application of the benzidine method to cells in which a centrosome is known to occur. The objects used were some of the most highly specialized cells, the spermatozoa of mammals. The animals used were the rat and the mouse.

(*a*) Spermatozoa.

In either case the testis was rapidly excised, cut across, a smear made, quickly hardened with sublimate, and washed with water for a few minutes. The benzidine was then applied diluted with an equal volume of glycerine. The stain did not take effect for several hours, and in some cases continued to deepen and bring out fresh oxidase masses in the cells for forty-eight hours afterwards..

In a smear of this kind the most conspicuous objects are the masses of mature spermatozoa. Isolated specimens of these are the first to show the characteristic blue of the stained oxidase. The head and part of the middle-piece of a rat spermatozoon, stained by this method, is shown in fig. 22, Pl. 39. The head has taken the stain, but it is pale and diffuse and is perfectly clear and transparent; there is no trace of oxidase in granules.

In the neck the two centrosomes with their connecting threads are clearly defined and stained, the proximal one paler, the distal one deep benzidine blue; the connecting strand of oxidase is slightly paler than the proximal centrosome. The middle-piece in the rat spermatozoon is extremely long in proportion and the whole of it is occupied by the spiral fibre. This is beautifully brought out by the benzidine blue, the staining is very sharp and well-defined. The proximal end of the spiral fibre is attached to the distal centrosome and the depth of stain is for the first few coils of the spiral as intense as in the centrosome itself. The intensity of the stain diminishes gradually and slowly towards the tail end where the depth is about the same as that of the stain in the proximal centrosome. In the figure only about one-half of the middle-piece is shown.

The head and middle-piece of the mouse spermatozoon give an exactly similar benzidine reaction. The two centrosomes seem to be relatively larger than they are in the rat and the depth of stain is more uniform. The spiral fibre is brought out just as clearly and as well defined as it is in the rat.

(*b*) Spermatocytes.

In the smears from the mouse testis a number of stages in the development of the spermatozoon were found. The stage shown in fig. 18, Pl. 38, represents a spermatocyte of the second order in an early phase. The preparation from which the drawing was made was stained first with benzidine and afterwards with picrocarmine. The appearance of the cell agrees very closely with that obtained for this stage in spermatogenesis by Lenhossek, Meves, and Benda. It is the stage described by them at which the divided centrosome is leaving the nucleus. In this particular cell this process of emigration is half completed, one of the pair of centrosomes, the distal one, is outside the nucleus and has affected the cytoplasm around it producing a slight radiation. The nucleus is stained diffusely with carmine, also the acroplast, and both contain a coarse, ill-defined irregular network of oxidase. At each side of the nucleus is a deeply stained body resembling a centrosome and stained with the benzidine blue even more deeply than the centrosomes. These bodies, which may be only transitory remains of the divided centrosome, have not been described by the above authors. The whole of the cytoplasm is filled with finely divided oxidase granules which has the appearance of a flocculent precipitate, not of a network. At the tip of the process forming the tail rudiment the oxidase is condensed into a kind of cap.

Fig. 19, Pl. 39, represents a spermatocyte in a more advanced stage of development. The nucleus still remains as a spherical body but its outline has become irregular, and in places the nuclear membrane is no longer definite, it has begun to degenerate. The nucleolus has lost its usual hard outline, and is only faintly stained by the benzidine. What oxidase there is in the nucleus shows only traces here and there of a reticulum; it seems to be inactive when compared with the cytoplasmic benzidine oxidase. The irregular distribution of oxidase gives an impression of nuclear disintegration. In the cytoplasm the benzidine oxidase is uniformly distributed and is well stained. There are large granules, of about the same size as the centrosome, scattered throughout the cytoplasm and forming the nodes of a coarse reticulum the strands of which are faintly stained but are easily seen, as their thickness is equal to the diameter of the granules. The reticulum does not extend into the part of the cytoplasm which is forming the middle-piece, but it is replaced here by a mass of oxidase of the typical benzidine blue colour which corresponds in position, size, and shape with the mass of mitochondria from which the spiral fibre is formed. At the distal end of the middle-piece the commencement of the spiral-fibre rudiment is clearly seen and consists of benzidine oxidase, rather more deeply stained than the main mass of mitochondria. The most conspicuous bodies in the cytoplasm are the two centrosomes. They lie just internal to the mass of mitochondria; in fact, the annular distal centrosome lies on the inner end of the mitochondrial mass. Both the distal and proximal centrosomes are stained very deeply by the benzidine, so that the blue tinge is almost lost as the depth of colour approaches black. In the tail the axial fibre has taken up the blue stain very slightly, and this is sufficient to show the root of this fibre arising from the proximal centrosome and passing through the ring of the distal one.

The same cell was stained with picrocarmine and is shown in fig. 20, Pl. 39, with the double stain. Almost the whole of the cytoplasm is suffused with the carmine stain, showing that the chromatin is no longer confined to the nucleus, in fact the nucleus is hardly stained at all by the carmine. The chromatin has at this stage become diffused as it is throughout the rest of the extent of the spermatozoon. As the drawing shows, no chromatin is in that part of the cytoplasm which is being formed into the middle-piece; in other words, it is confined here, as it is later, to the head. This portion of the cytoplasm and the remains of the nucleus itself appear to be the only parts containing no chromatin. The pink stain helps to bring out more distinctly the coarse reticulum of oxidase as a comparison of fig. 20 with fig. 19, Pl. 39, will show.

At the stage of spermatogenesis just described, the process of cytoplasm forming the middle-piece extends only a very short distance along the axial fibre. It seems to extend in figs. 19 and 20, Pl. 39, as far as the last turn of the developing spiral fibre. Beyond this point the axial fibre is apparently bare of protoplasm. At the stage reached by the developing spermatozoon shown in fig. 21, Pl. 39, the protoplasm of the middle-piece has spread and thickened over the axial fibre. The characteristic shape of the head is now appearing and the curved sharp point is being formed by the constriction between the head and a mass of protoplasm which is being divided off. This mass contains diffuse chromatin and oxidase. The process which gives the head its characteristic shape has all the appearance, at this stage, of having grown out laterally and of being a purely cytoplasmic process from the axial mass, containing no chromatin and only a little oxidase more or less diffused. The position of the two centrosomes in relation to the neck is not that which they finally take up, and this indicates that this stage is quite an early one in the formation of the head. In the fully developed spermatozoon the centrosomes lie in the very slender neck placed opposite the recurved portion of the head. In fig. 21, Pl. 39, the proximal centrosome is placed well in front of the neck, and the neck itself is extremely thick. It looks as though the two centrosomes were carried backwards, as the protoplasm draws out to the fine thread just thick enough to contain the two centrosomes in the fully developed neck. The three processes in the formation of the head and neck, just described, point to the conclusion that the protoplasm at this stage is in such a state of activity as might be called pseudopodial. The separation of the extruded mass from the point, the formation of the lateral mass of the head, the thinning of the neck, and also the extension of protoplasm along the axial fibre, are four movements of protoplasm, all four taking place simultaneously, and all being of the nature of a growing pseudopodium.

In that part where the middle-piece is forming, the oxidase is taking the shape of the spiral fibre at some distance from the centrosome. Between the spiral coils and the distal centrosome are irregular masses of oxidase without any spiral arrangement. The four or five spiral coils visible were already formed at the stage shown in figs. 19 and 20, Pl. 39, where they are seen to lie quite close to the centrosome so that the movement of the protoplasm has evidently carried them away from the head, while the mass of oxidase lying between the spiral and the centrosomes has considerably increased in bulk. The distal centrosome is still ring-shaped and a faint band of oxidase forms a broad connexion between the two centrosomes.

(*c*) The Centrosome in Mitosis.

The centrosome has long been known to bear relation at the same time to cilia and to the spindle of a mitotic figure, in the first as a basal body and in the second as a centriole. In the dividing spermatocyte of some Lepidoptera, Meves (1900), Henneguy, and Gatenby have described the existence of cilia arising from the single or paired centrosome at the poles of the spindle (figs. 26 and 28, Pl. 40). In the living spermatocyte of Eschistus, Montgomery (1911) has seen the formation of a pseudopodium (fig. 25, Pl. 40) in a position corresponding to that of the cilia in Bombyx (fig. 28, Pl. 40), at the surface lying over each end of the mitotic spindle. The flagella of the dividing collar-cell of Clathrina coriacea (Montagu) are also known to grow out from the centrosomes of the cell (fig. 27, Pl. 40, after Robertson and Minchin, 1910). If the ciliary or pseudopodial movements are in some way caused by, or related to, the activity of the oxidase, it seemed likely that the movements of the various structures in cell-division would also be related to oxidase activity. Some of the essential parts of the cell in mitosis and especially the centrosome would be found therefore to contain oxidase.

As the gonocytes in the mouse are smaller than those of the rat, the smears of the mouse were not searched for dividing cells, but similar smears were made from rat testis. In these smears a few dividing spermatocytes were found, and two of these are shown in figs. 16 and 17, Pl. 38. In fig. 17, Pl. 38, a primary spermatocyte is shown at the end of the prophase of mitosis and stained by the benzidine method. The cell contains much scattered oxidase in large and apparently isolated granules without connecting network, but the most striking feature of this cell is the stained mitotic figure. The sixteen threads of the spindle could easily be counted and traced from the equatorial plate to the poles, where they ran into the deeply stained mass which represents the central body of the astrosphere. This body included the centrosome, and at the upper pole in the figure, which is turned towards the observer, the centrosome would have been visible if it had stained either more or less than the surrounding astrosphere. There was, however, no difference to be seen. At the equatorial end of each spindle-fibre there was a dark mass of deeply stained oxidase arranged in pairs in such a way that they seemed to envelop the divided chromosomes on the point of moving away from the equatorial plate towards the poles.

Fig. 16, Pl. 38, represents an early prophase of the same stage in spermatogenesis. The cell is judged to be at this stage as its size corresponds exactly to that shown in fig. 17, Pl. 38. The stage which has been preserved in this cell is the first step in the formation of the spindle after the bipolar position has been taken up by the centrosomes. The whole cell is filled with a reticulum formed of oxidase. The size of the rather more deeply stained granules at the nodes is on the whole rather less than those in the spermatocyte shown in fig. 19, Pl. 39, although some of the granules, especially those lying on the nuclear membrane, are quite as clear. In shape the granules are irregular spheres, and it may be that those which appear larger on the nucleus are slightly flattened out. Two deeply stained masses of oxidase indicate the position of the centrosomes at the poles of the future spindle. The centrosomes themselves are not more deeply stained than the rest of this mass and can accordingly not be distinguished. Radiating out from this mass, as a centre, in all directions, are more deeply stained strands of oxidase giving to the mass and its rays an appearance of the typical astrosphere with coarse, thick, and short radial processes. In the lower astrosphere of fig. 16, Pl. 38, the formation of the rays has gone farther than it has in the upper one, and two long straight strands of oxidase can be seen passing from the astrosphere to the equatorial plane of the nucleus. The nucleus is not yet orientated and lies eccentrically in the cell; the centering of the nucleus will be brought about as the spindle develops and the centrosomes reach the poles of the cell.

CHAPTER XIII.—DISCUSSION OF RESULTS

A. PSEUDOPODIA, CILIA, AND FLAGELLA.

THE morphology of pseudopodia, cilia, and flagella is not sufficiently known to enable any assertion to be made as to the derivation of one of these motor organs from either of the other two, when considering any one organism. However, there is no doubt that the pseudopodium is the most primitive of these three organs, and it is generally accepted that the pseudopodium is the structure from which flagella and cilia have arisen phylogenetically, as they are known to do so ontogenetically. Granting this hypothesis, the morphological correspondence carries with it in this particular instance the implication that the motor activity of all three organs is due to conditions which apply to all equally. That this must be so is perfectly clear from the few known observations of a pseudopodium becoming a flagellum or a cilium, and of a flagellum becoming a pseudopodium. It follows from this that if the conclusion arrived at in Part I (p. 595) is correct, namely that the movements of pseudopodia are conditioned by oxidase action, the movements of flagella and cilia are similarly due to oxidase action. It would therefore be a confirmation of the above hypothesis, of the most valuable kind, should each flagellum and each cilium of cells from different phyla be found to be connected with an oxidase organ.

The observations recorded above are, I think, sufficient to substantiate the claim that the motility of pseudopodium, flagellum, and cilium is based on a physiological agency which is the same in all cases. The functioning of oxidase as applied to pseudopodial movement (see Part I, p. 595) will then equally well apply to the movement of a flagellum or a cilium with the difference that this functioning is in the last two cases more organized, and for the cilia furnished with some co-ordinating system.

It will be simpler to deal with the cilia first as the modus operandi is somewhat clearer than it is in flagella. In Paramecium each cilium has in its basal body a source of energy, the Kinetosome. The same method of converting the chemical energy of the oxidase action into the energy used in movement applies here as it did in Arcella; each basal body acting on the surface of the protoplasm in the cilium, and owing to its minuteness, each basal body acts only on its own cilium. Normally the cilia in Paramecium all bend in the same way which may roughly be described as an inclination of the cilium backwards. On the oxidase hypothesis, this movement is due to a softening of a patch of ectophragm on the posterior surface of the base of the cilium. The softening is brought about in exactly the same manner as it is in the production of pseudopodia in Arcella. It is due to electrolytic changes in the oxidase system and consequent alterations in the Helmholtz double layer of the patch of ectophragm owing to the accumulation of ions and changes in the chemical equilibrium at the surface.

One of the striking characteristics of a living ciliate is the more or less complete co-ordination of the ciliary movements. Commencing at the anterior end a band of bent cilia, almost at right angles to the long axis, appears to move back to the posterior end. In reality the cilia in the middle of this band are at the bottom of their stroke, those at the anterior edge of the band are recovering, and those at the posterior end are commencing their stroke. Verworn's experiment on Spirostomum showed that the co-ordinating impulse travels in a line roughly parallel to the long axis, for the slight notch which he made in the surface of one of these Polytrichids caused the movement of the cilia placed immediately behind the notch to cease. On the hypothesis that at each beat of a cilium oxidase action is suddenly started or intensified in its basal body, the spreading of this action along the strands of oxidase radiating from the basal body will extend along those strands which have not already been activated. The activated strands must necessarily always be the one or two which pass into the basal body from the anterior end, since the cilia on that side have just moved. The reason why the current of oxidase action must always pass in one direction is at once provided by the hypothesis, according to which an electrical tension is set up on both oxidase and ectophragm, and this tension so long as it lasts at its height puts a stop to any further oxidase action in the excited area. It was pointed out in Chapter XI of this paper (p. 602) that the interaction of oxidase and ectophragm is reciprocal. Bearing this in mind, the transmission of an impulse from the anterior to the posterior end of Paramecium by the agency of the oxidase network can be explained without difficulty. When the basal bodies at the extreme anterior end stimulate their cilia by a stream of kations, the latter must be radiated from each basal body, in such a way that the cross-section of the stream will be greatest where it impinges on the ectophragm. The area of ectophragm thus excited will be greater than the area covered by the oxidase of the basal body and a portion of it will overlap a part of the oxidase strand connecting the basal body with the next posterior one. The overlapping excited portion of the ectophragm will stimulate the part of the oxidase strand immediately below it and this will again radiate kations. The stream of kations will be deflected from the normal towards the posterior end because the surface lying opposite to it has already been positively charged by the initial stream from the first basal body. In this way the stimulation of the ectophragm will always be in advance of the activated part of the oxidase network, and in this way the passage of excitation of the cilia from the anterior to the posterior end can be explained. This explanation must be harmonized with what is known of the action of galvanic electricity on ciliary movement and the influence of electrolytes in the surrounding water.

When describing the oxidase network in Coleps hirtus (p. 612) the widely separated basal bodies of the cilia were shown to be connected by a network containing what were regarded as being vestigial basal bodies. Coleps is admittedly an armoured representative of the large order of Holotricha all of which, with this and one other exception, are unarmoured and covered with a continuous, evenly distributed coat of cilia. The two hundred cilia which Coleps possesses are confined each to a patch of protoplasm left uncovered by the armour plates, the majority of them are inserted between the edges of two adjacent longitudinal rows of plates, that is along lines parallel to the long axis. These longitudinal rows of cilia, so far as their movements are controlled by the oxidase network, must beat independently of each other, for there can be no impulses passing through this network from one row to another. The armour plates being solid prevent the formation of a Helmholtz double layer on the ectophragm in contact with them, so that impulses pass only along the network lying below the notched edge of each row of plates.

In both Paramecium and Coleps, the whole of the protoplasm is permeated with a network of oxidase strands thickened at the nodes and continuous throughout cytoplasm and nuclei. This network forms a part of the macronuclear and micronuclear membrane and the network inside both nuclei has meshes of the same size as in the cytoplasm. The only difference which applies both to Paramecium and Coleps is that the micronucleus has in both genera larger and more sharply defined nodes than the macronucleus and the cytoplasm.

The ciliated cells of the nephrostome in Lumbricus (fig. 15, Pl. 38) have an internal network remarkably similar to that of the two infusoria just described. In these cells, however, the network is even less differentiated than in the Ciliates, as the size of mesh and of the nodal thickenings is the same in cytoplasm, nucleus, nucleolus, and so far as could be made out in the rather thick preparation, in the karyosome.

The network of oxidase showed exactly the same character in the metazoan ciliated cell and in the two ciliates, whether the killing and hardening was in cold saturated solution of corrosive sublimate or in 4 per cent. formaldehyde solution. If Hardy's results (1899) on the coagulation of the protoplasm in gut cells of Oniscus apply to such cells as are now being dealt with, the uniformity of the meshwork in such widely separated cells would indicate a most remarkable similarity of protoplasmic structure. This similarity would extend in Lumbricus for instance throughout the cytoplasm, nucleus and nucleolus and, as the coagulation of these structures is undoubtedly not the same, it follows that the network of oxidase as seen in preserved specimens is not to be regarded as a post-mortem arrangement of oxidase in the coagulum conditioned by the process of preservation. It does not follow, however, that the network of oxidase exists in the living cell exactly as it is seen in the preserved specimen.

B. CENTROSOME.

The step from basal body to centrosome is a short one. Henneguy and Meves found that the centrosomes of Bombyx function both as centrosomes of the mitotic spindle and the basal bodies of flagella in the dividing spermatocyte. On the strength of this it was obviously to be expected that the benzidine reaction would differentiate the centrosomes of mammalian spermatocytes. This expectation was fulfilled and it was found that the stain in the centrosome of the spermatocyte was deeper and more definite than in any other of the oxidase structures brought out by treatment with benzidine. In addition to the centrosomes the developing spermatozoon (figs. 19 and 20, Pl. 39) contains oxidase in two other forms, one deeply stained like the centrosomes and scattered through the cytoplasm, the other stained light blue, scattered in the nucleoplasm and massed in the rudiment of the middle-piece. All the scattered oxidase both in nucleus and cytoplasm seems to vanish (fig. 21, Pl. 39) even before a large proportion of it is cast out by the separation of the anterior part of the head. This apparent disappearance of oxidase at this particular stage of spermatozoon development is of great interest and will be referred to later when discussing the inertia of the unfertilized ovum.

C. Mitochondria.

The fate of the mass of oxidase in the middle-piece, in forming the spiral tract in this part shows that this mass of oxidase is probably identical with the mass of mitochondria described in this position by Benda, Meves, and others. This identification of chondriosomes with oxidase-substrate seems to me to be of the greatest importance.

It has been clearly established by those who have studied the spermatogenesis of the animals which possess a spiral band in the middle-piece of the spermatozoon, that this spiral band is formed from chondriosomes. It seems beyond doubt, after what we have learnt of the source of movement in pseudopodia and cilia, that the flagellar action of the spermatozoon tail is to be attributed to the action of the oxidase in the spiral band or filament of the middle-piece. This spiral band is formed from a certain number of the chondriosomes only, which may be an indication that there is either a difference in kind between these particular chondriosomes and the others contained in the spermatocyte; or that the chondriosomes pass through a series of changes which make it possible for them at different stages to behave differently, and perform different functions in the cell.

When discussing the structure of the oxidase-substrate granules, it was pointed out that the main bulk of these granules consisted probably of a substrate, and that the oxidase portion of the granule was extremely minute. It was also pointed out that the oxidase granule, in order to be activated by OH-ions, must be situated on the surface of the bulky substrate. The arrangement is thus anisotropic. If the chondriosomes have the same anisotropic structure, it is easy to explain on this basis why, at certain stages in the activity of the cell the chondriosomes form long threads or chains—chondrioconts (Meves), chondriomites (Benda), mitochondria (Montgomery). When the cell containing chondriosomes has passed through a stage of activity during which the respiration of the cell has been proportionately increased, the oxidases will have used up the substrate more or less, and the polarization both of the substrate and the oxidase portions will be less pronounced. The charge of the whole granule will be of opposite sign at either end, the substrate positive and the oxidase negative; and the result will be that those chondriosomes which closely approach each other will attract each other electrostatically; and if two granules have joined end to end, i. e. substrate end to oxidase end, the coalesence will further reduce the charge, and the positive end of the short filament will attract the negative end of single chondriosomes, so that the filament will constantly tend to become longer and longer. The charge on the spindle-threads of the mitotic figure being positive, as it consists of oxidase filaments with the substrate external, it might be expected that the long mitochondrial filaments would arrange themselves parallel to the mitotic figure in a divided cell; because the bulk of the filament consists of substrate which, like the spindle-threads, has a positive charge. This arrangement of spindle-threads has been observed by Meves, Benda, Montgomery, and Gatenby. Any cell which has been actively functioning and passed into a resting stage, and which contains mitochondrial filaments, may again become active, and the polarization of some of the filaments will be raised in potential. The coherence of the chondriosomes under the increased potential may be insufficient to hold the thread together, with the result that it will break up into the original chondriosomes, as a fine jet of water breaks up into drops, as soon as the jet is electrified.

D. FERTILIZATION.

The observation that the centrosome contains or consists of oxidase contributes a new fact to the solution of the problem of fertilization. The entrance of the spermatozoon into the ovum fulfils two main functions. It introduces the substance or structure which conveys to the zygote the power to reproduce paternal characters, and it introduces some element which rouses the matured ovum from its resting condition and initiates the process of segmentation. This process may be said to commence when the centrosome of the spermatozoon begins to form the aster which, after a short time, surrounds the fused pronuclei and then becomes the first diaster of the segmenting egg. As the experiments on Arcella, especially those with vital stains, distinctly showed that a radial structure in protoplasm is one of the features which accompanies oxidase action, so do we get in the impregnated ovum a radial structure of protoplasm formed round the introduced fragment of oxidase in the centrosome. This radiation in the segmenting egg is transformed into the first segmentation spindle, and as shown in fig. 17, Pl. 38, the spindle of mitosis is formed from or contains oxidase. With these facts it is evident that the introduction of the sperm centrosome or centrosomes is the factor which determines the commencement of cell-division after fertilization.

Formation of polar bodies.—Those cell-divisions which lead to polar body formation besides reducing the chromatin also reduce the quantity of oxidase in the ovum, for each polar body receives the oxidase of half a mitotic spindle contained in the centrosome, astrosphere, and spindle fibres. This oxidase reduction may well be one of the factors which determine the inertia of the resting mature ovum, but it is not the only factor. The very fact that the cell-divisions which produce the two polar bodies, where they take place before the spermatozoon has penetrated the egg, do not give rise to equivalent cells shows that the proportion of oxidase left in the mature ovum is only a very small fraction of the amount which would enable a cell of the size of the ovum to undergo equal segmentation. The second polar body and the ovum each contain one quarter of the oxidase present in the oocyte of the first order. Even in the minute polar body this quantity seems to be insufficient to bring about further cell-division whereas in the first polar body which contains twice as much oxidase, one division by mitosis is possible and often takes place. The oxidase introduced into the ovum by the spermatozoon is at least equal to that already present, and where the middle-piece contains a large spiral of mitochondria the introduced oxidase will greatly exceed that of the ovum. When the first segmentation spindle is formed it may safely be taken as consisting of both oxidase derived from the ovum and from the centrosomes and the mitochondria of the spermatozoon.

Another factor of equal if not of greater importance than the amount of oxidase in the ovum is the manner of its distribution, or what might be called the oxidase organization of the cell. When, in a holoblastic egg the first polar body is formed, the mitotic figure is peripheral and the organized oxidase does not extend in the rays directed internally beyond the centre of the ovum at the most. At least one half of the ovum contains no organized oxidase and remains quite inert during cell-division. But when the first segmentation is set about in a holoblastic egg, the arrangement of the segmentation spindle is central and the oxidase in the asters may and often does spread through the whole of the cell. This approaches the relation of spindle to cell in a normal somatic mitosis. The importance of organization in the cell-elements was recognized and experimentally proved by Warburg (1913). He showed that when the egg of Echinoderms was broken up, a stage was reached, when the fragments were reduced to a certain size, at which the oxygen exchange ceased, while before that stage was reached the amount of oxygen taken up by the fragments of protoplasm remained practically undiminished. The conclusion was drawn that the respiratory absorption of oxygen by the cell depends on the cell structure, and that the amount consumed is roughly parallel to the amount of structure which is preserved in the fragmented egg. This result is perfectly compatible with the cytophragm hypothesis. When the egg in this experiment is reduced to smaller and smaller fragments, there is an artificial production of an enormously increased ectophragmal surface ; but at the same time the organized relation of oxidase to the original ectophragm is gradually being destroyed.

The meaning of Warburg's results would seem to be that in the intact egg the structure is, on the oxidase theory of respiration, extremely highly organized. As the respiratory activity of the unfertilized egg is comparatively speaking extremely low (Shearer 1922), it follows that the organization is not completed until after the entrance of the oxidase carried in by the spermatozoon. The amount of oxidase introduced stands in no proportion to the enormous rise of oxygen consumption. Carrying this argument a stage further, we arrive at the conclusion that the maturation of the ovum in its final stages, i.e. just before and during the expulsion of the polar bodies is, to a very large extent, a matter of the efficient organization of the oxidases.

E. CELL-DIVISION.

Another side of the organization of the intracellular oxidases is that concerned in the act of cell-division itself. The forces at work in this process have hitherto been considered from many points of view without any general agreement being arrived at as to their nature. Divergence of opinion has gone so far that it has even been possible for one cytologist to invent a new force, mitokinetism, which comes into play when cell-division by mitosis takes place. One of the results of the present investigation will be to show, when the subject has been worked out in greater detail, that the actions and reactions brought about by the activity of the oxidase in the mitotic figure are sufficient to account for the movements of protoplasm in the dividing cell.

Before the whole process of cell-division can be reduced to terms of oxidase activity it will be necessary to trace the distribution and action of the oxidase in all the phases of mitosis. But it is already safe to say that the movements of the dividing cell as a whole and of its contents may be quite as adequately explained by the oxidase cytophragm hypothesis as were the pseudopodial and ciliary movements in Protozoa.

F. Artificial Parthenogenesis.

In the light of these results the problem of artificial parthenogenesis assumes a different aspect. If fertilization is regarded as the initiation of the process of segmentation or cell-division, it is then easy to see that the stimulation of the ovum oxidases by any agent may take the place of the stimulation by the oxidase in the centrosome of the spermatozoon. Varied as the methods of artificial parthenogenesis are, they can, I believe, be reduced to this one common denominator.

This is not the place to discuss in full the subject of artificial parthenogenesis, all that will be attempted here will be to take in order the chief methods which have been used to stimulate the development of the egg without impregnation by a sperm of the same species.

These methods can be grouped under two heads: (1) indirect stimulation of the oxidase through ectophragm; and (2) direct stimulation of the oxidase without the intervention of the ectophragm. The first group includes the methods of Tichomiroff, Loeb, Delage, &c. The second group comes under three heads, the oxidase may be stimulated directly either (*a*) by reducing the oxygen partial pressure and then restoring it, or (*b*) by temporary dessication (Giard), or (*c*) by the introduction of oxidase into the egg.

1. The method of artificial parthenogenesis by stimulation of the ectophragm will be discussed in full, it is hoped, in a later paper. On the hypothesis that there is reciprocal action and reaction between ectophragm and oxidase endophragm in the cell,

it is easy to see that stimulation of the ectophragm in a mature ovum may, in favourable cases, cause the oxidase to react.

2. The methods included in the second group mentioned above bear more directly on the subject of the present investigation.

The third method (*c*) of direct stimulation of the oxidase in the ovum in artificial parthenogenesis can be effected in two totally different ways. The introduction of the spermatozoon of a different species, genus, order, or even of a different phylum is, according to this theory of oxidase stimulation, one of these methods. The active element in this kind of fertilization is, on this view, the oxidase of the foreign spermatozoon which, after its introduction into the ovum, is associated with its own substrate and surrounded by a portion of its own cytoplasm, and is thus able to form an aster. While the aster is being formed the centriole of the ovum is stimulated, and forms a second aster. The effect of the formation of the foreign aster on the oxidase and on the electrolytes of the ovum must be exactly similar to the effects produced by an aster formed by the activity of an oxidase derived from a spermatozoon of the same species. We know that under these normal conditions the two asters keep pace in their development to a remarkable extent. The activation of one part of the oxidase of a cell by the activities of another part, has already been illustrated by several examples in Arcella and in Ciliates.

The method of artificial parthenogenesis which has not yet been discussed is the method of Bataillon (1913). This method in its final form is the stimulation of the ovum by the introduction into an amphibian egg of a mammalian leucocyte. That the leucocyte is actually carried into the ovum was proved by Herlant (1913). That such leucocytes contain benzidine oxidase is well known, and to the action of this oxidase the stimulation of the oxidase in the ovum must be attributed. In Herlant's figures, where the aster of the ovum is developed, one or more asters are shown to be in the immediate neighbourhood of the leucocyte. Whether these asters are derived from the oxidase of the leucocyte or not is not certain. The presence of an aster in the neighbourhood of the leucocyte may possibly be accounted for in another way : it might be the result of a condensation or activation of oxidase already existing in the cytoplasm of the ovum under the influence of the activity of the surviving oxidase in the leucocyte. On the whole I am inclined to consider that the treatment of the leucocyte which is forcibly pushed into the cytoplasm of the ovum by the needle, is sufficient to destroy the cytophragm of both, leucocyte and ovum where they come into contact, and force some of the oxidase of the leucocyte into the cytoplasm of the ovum. Even if the leucocyte is seriously damaged, the action of its oxidase will not be interfered with, for oxidase activity has been shown to continue unchecked in minute fragments of the protoplasm.

CHAPTER XIV.—CYTOPHRAGM HYPOTHESIS AND MUSCULAR CONTRACTION.

In two of the systems of organs of the Metazoa, viz. the muscular and the nervous system, the functioning of the tissue-cells is accompanied by marked polarization phenomena. If the cytophragm hypothesis is to serve in working out the nature of muscular contraction and nervous impulse, the first step in bringing these functions into line with the observations recorded above would be to show that oxidases play a fundamental part in muscle, and nerve-cells, when they respond to stimulus.

The attempts made to demonstrate oxidases in muscle-cells by colour reaction are not likely to be successful, because the very powerful reducing agents present in the cell always tend to counteract the oxidation of the colour base. Hence the proof of the presence of oxidase must necessarily be indirect and is based on the assumption that chondriosomes correspond probably to the oxidase-substrate granules.

It is therefore important to make out whether in the structure of the muscle-cell the chondriosomes are arranged in such a way as to assist in producing the reactions found in contraction.

One method of proof is the observation of what enters into the composition of the muscle-cell during its development. This method already supplies evidence that the organization characteristic of muscle-cells is mapped out in the developing muscle fibrillae of the chick, by the banded appearance shown in the arrangement of the chondriosomes. [Duesberg (1909–10).]

If the cytophragm hypothesis is to explain muscular contraction on lines suggested by the explanation of pseudopodial and ciliary movements, and thus bring all the methods of animal movements under one scheme, the philosophical way of looking at the evolution of a given structure or function requires that in a proof of this kind it should be shown how a lower form of structure or function can change gradually into a higher form. When dealing with movement, the least highly organized is pseudopodial movement, and the highest is muscular.

One instance is known of a remarkable change in a cell from amoeboid movement to muscular contraction. In Tergipes dispectus C. B. Wilson (1898) observed the development of two large mesenchyme cells which are at first spherical and floating freely in the segmentation cavity of the highly transparent veliger larva. They then become amoeboid, and actively put out branching pseudopodia, and except for their size, they resemble the ordinary stellate mesenchyme cells. The branching cells then cease to put out pseudopodia and 'pulsate in more or less rhythmical contractions', until the ends of some of the pseudopodia attach themselves to the enteron and to the outer body wall. After attachment the two cells act as muscle-cells. The change from amoeboid cell into muscle-cell takes place in the course of a few hours. The point of interest is the rapid change from the amoeboid to the contractile state. The period of pulsation may be regarded as the final stage of the amoeboid condition, as it consists in the alternate extension and retraction of large areas of the surface; and such extended areas may be interpreted as the protrusion of a broad but short lobopod. On the hypothesis of the production of pseudopodia by an oxidase-endophragm, the oxidase is, during the amoeboid stage, functional in small patches, thus leading to the protrusion of typical pseudopodia. During the pulsating stage the oxidase is functioning uniformly over large areas, and is at the same time gradually rearranging its distribution by a process similar to that already described in the formation of chondriosome filaments (p. 627). This process consists in the arrangement of the oxidase substrate granules firstly as long chondriosome filaments, which secondly become parallel to each other as a result of the action of the surface potential. This action is the same in the arrangement of the muscle fibrillae as it is in the arrangement of the chondriosome filaments of the dividing cell, which lie roughly parallel to each other, and parallel to the spindle fibres of the dividing nucleus. In some such manner as this the condition described by Duesberg (1909–10) in the embryonic muscle-cell is reached, where the chondriosomes are distributed in such a way as to mark out the fine structure of the fibrillae of the future muscle-cell.

While the arrangement of the chondriosome (oxidase) just described is taking place, and as soon as long chondriosome filaments (of oxidase-substrate) are formed near the surface of the cell, a number of the filaments, acting together as an endophragmal surface, would produce by their influence on the ectophragm, the broad short lobopod, the rhythmic protrusion and retraction of which would produce the appearance of pulsations. These would cease, and muscular contraction would commence as soon as the chondriosome filaments assume the relations to each other which would give the whole cell or parts of it the structure of bundles of muscle fibrillae. At this stage the pulsations would stop, and muscular contraction begin.

CHAPTER XV.—CYTOPHRAGM HYPOTHESIS AND NERVE FUNCTION.

A. Development of Nerve Processes.

On fig. 23, Pl. 40 a series of drawings are given which have been copied from a classical paper by Harrison (1910). They represent the growing end of a nerve-fibre in a culture of surviving tissue, grown from the spinal cord of a frog embryo. For the forty-seven minutes during which growth was progressing through the seven stages figured, the tip of the fibre was in constant amoeboid movement; the pseudopodia being of two kinds, and very similar to the marginal pseudopodia of Arcella. There are comparatively broad lobopods bearing fine filopodia; from their appearance it may be inferred on the cytophragm hypothesis, that there is an oxidase endophragm in the growing tip of the fibre. Hoven (1910) has demonstrated that the so-called neuro-fibrillae of the developing nerve in the embryonic chick, are formed by the arrangement in threads of the chondriosomes in the nerve-fibre. On the cytophragm hypothesis, the oxidase granules, which act as endophragm in producing amoeboid movement, would be available, as they increase in number during the process of growth, for the production of the train of oxidase constituting the axial strand of the nerve-fibre. The action of such a strand of oxidase in conducting nerve impulses would thus be similar in every way to the action of the connecting strands of benzidine oxidase between the basal bodies of cilia in the protozoan and metazoan cells which have been described (p. 607).

B. Nature of the Nerve Impulses.

Under the cytophragm hypothesis the nature of the nerve impulse consists in the progressive oxidation by the oxidase granules in the axial filament of a substrate.

The experiments of Winterstein, Verworn, and others, on the importance of oxygen in maintaining the nerve function, are I believe explained on the hypothesis that the oxidase of the axial filament is an essential element in the conduction of the impulse. In the most efficient form of nerve known, the 'medullated' nerve, surrounding the inner axis is a sheath containing myelin, the functional part of which consists of substances regarded as organic soaps. According to the cytophragm hypothesis, the presence of this alkaline myeloid substance forms a medium containing hydroxyl-ions, in contact with and surrounding the ectophragm of the protoplasm of the nerve process, the endophragm consisting of the axial strand of oxidase.

The passage of a nerve impulse on this hypothesis can be compared with the passage of combustion along a train of gunpowder. The oxidase strand represents the explosive, and, like it, requires oxygen along its whole length in order that the explosion may travel. The speed of a nerve impulse is of the same order as the rate at which an explosion travels along a tube of a mixture of explosive gases, i. e. it travels at the rate at which chemical combination is propagated along a suitable train of matter on the verge of combining under the influence of a minimum stimulus.

The comparison with a train of gunpowder can be carried farther. To both, the 'all-or-none' principle applies equally well. Moreover, could we imagine the train of gunpowder passing at a short part of its course through an atmosphere deprived of oxygen, the passage of the explosion would be blocked at this place. A similar block is shown to occur in a nerve under conditions affecting a short length of it which prevent oxidase action in one way or another by the treatment applied to that length.

The nerve impulse in a motor nerve originates in a ganglion cell. Where the ganglion cell is in a synapsis, the stimulation of the cytophragm is conceived to be its polarization induced by the polarization of the end network of its stimulating nerve-fibre. This conclusion, so far as the polarization is concerned, although arrived at by a totally different method, is identical with that suggested by Bayliss (1918). The suggestion made by Bayliss, p. 471, that the polarization of the dendritic nerve-endings in a synapsis is the means of stimulating a similar nerve-ending or a ganglion-cell, falls into line with the suggestion made above, if it is further considered that the permanent branching processes of a nerve-ending may be and probably are amoeboid, like those amoeboid processes seen on the growing-point of an embryonic nerve by Ross Harrison (see fig. 23, Pl. 40). In both of these instances of amoeboid movement the pseudopodia are formed as the result of the activity of the oxidase known to be present.

C. THE TROPHIC ACTION OF NERVES.

One of the consequences of accepting this view of the nature of the nerve impulse would be that it explains how the passage of such impulses, either to or from a nerve-ending, can exercise a trophic action on the tissues either supplied by a motor nerve or contained in the endings of a sensory nerve. The result of nerve action involves in each case a polarization of the nerve-ending, and as this polarization is on the hypothesis due to oxidase activity in the nerve, the polarization will be of such a magnitude as to bring about the polarization of the cells in the tissue supplied by the nerves. Such an effect would lead, by the reciprocating action of ectophragm and oxidase, to the stimulation of the oxidase in the tissue-cells. Where the oxidase in such cells is in a condition, and has relations to the cell, similar to that obtained in a mature ovum, the induced polarization of such a cell by the nerve-ending will stimulate the oxidase to form, as it does in the stimulated ovum, astrospheres which initiate the process of cell-division. The well-known experiment of Head and Rivers (1908) showed among other things that the regeneration of a scar on the hand commenced when the regeneration of the gap allowed sensory impulses to pass. The replacing of the scar tissue is due to the proliferation of uninjured cells round its edge by processes of cell-division, and these processes, as demonstrated by the benzidine tests, are carried out by the organized functioning of the cell oxidases in the formation of the mitotic figure, and the process of mitotic cell-division. The polarization of the nerve-ending being the consequence of oxidase activity is sufficient to give the required stimulation of the oxidase in an adjoining tissue cell. If the hypothesis of the oxidase nature of the axial fibre of nerves is used to explain the passage of a nerve impulse, it would be expected that the movement of K-ions would play an important part in the process. Macallum's work on the distribution of potassium in the axis cylinder of vertebrate nerves shows that potassium is present in abundance in the axis cylinder, and that it tends to collect at the end of segments of the nerve; i. e. on either side of a node of Ranvier, where the axis cylinder is in contact with the medullary sheath. The experiments of J. S. Macdonald (1902–5) show that movement of the K-ions actually does take place in a stimulated nerve. When trying to explain ciliary movement on the cytophragm hypothesis, a wave of K-ion concentration in the cytophragm was supposed to be one of the features of the passage of (*a*) a conduction impulse along the connecting strand of oxidase, and also (*b*) the motor function of the basal body. As the conducting and motor mechanism are of the same nature, i. e. oxidase action, the basal bodies conduct the impulse in the same manner as the connecting strands. Regarded in this light, a single line of oxidase strands connecting the basal bodies of an antero-posterior row of cilia in the superficial network in any ciliated cell, can be compared with the axis fibre of a metazoan nerve.

In Macdonald's experiments on injured nerves, the 'surging' of K-ions may be compared with the supposed wave of K-ions passing along such a line of conduction in a ciliated cell.

It is of interest to note that Nernst (1899), and following him Keith Lucas (1910), and A. V. Hill (1910), pointed out that in the electro-motor explanation of the nerve impulse, the theory demands the presence of two polarized surfaces (membranes). On the cytophragm hypothesis as applied to nerve, these two surfaces are: (1) the surface of the oxidase strand of the axis cylinder; and (2) the surface of the axis cylinder itself, where it is in contact with the medulla sheath. The presence of organic soaps in the medulla outside the surface of the axis cylinder, ensures the maintenance of an alkaline (OH-ions) medium, external to the cytophragm of the axis cylinder, while the K-ion concentration on the inner side of this membrane serves as the fluctuating anion component of the double layer of the cytophragm.

REFERENCES.

Andrews, E. A. (1897).—" Spinning in Serpula eggs ", 'Am. Nat.', **31**, 818–20.

—— (1898).—" Filose activity in Metazoan eggs ", 'Zool. Bull.', **2**, 1–13.

Bataillon, E. (1913).—" Démonstration définitive de l'inoculation superposée à la piqûre en parthénogenèse traumatique ", 'C.R. Ac. Sc. Paris', **156.**

Bayliss, W. M. (1918).—'Principles of General Physiology', 2nd ed. London.

Bütschli, O. (1875).—" Zur Kenntnis der Fortpflanzung bei Arcella vulgaris Ehr.", 'Arch. f. mikr. Anat.', **11**, 459–66.

—— (1880–2).—Bronn's "Klassen und Ordnungen des Thier-Reichs ", **1.** 'Protozoa.'

Delage, Y., et Hérouard, E. (1896).—' Traité de Zoologie Concrète ', **1**, 438.

Duesberg, J. (1909).—" Über Chondriosomen und ihre Verwendung zu Myofibrillen beim Hühnerembryo ", 'Verh. d. Anat. Ges. zu Giessen.'

—— (1909).—" Les chrondriosomes des cellules embryonnaires et leur rôle dans la genèse des myofibrilles ", 'Arch. f. Zellforsch.', **4.**

Ehrlich, P. (1885).—' Das Sauerstoffbedürfniss des Organismus.' Berlin.

Engelmann, T. W. (1869, i).—" Beiträge zur Physiologie des Protoplasma. 1. Ueber periodische Gasentwicklung im Protoplasma lebender Arcellen ", Pflüger's 'Arch. f. d. ges. Physiologie', **2**, 307.

—— (1869, ii).—" Sur le développement périodique de gaz dans le protoplasma des Arcelles vivantes ", 'Arch. néerland. d. sc. ex. et nat.', **4**, 424–30.

—— (1869, iii).—" Sur l'irritation électrique des amibes et des Arcelles ", ibid., 431–2.

—— (1878).—" Ueber Gasentwicklung im Protoplasma lebender Protozoen ", 'Zool. Anz.', **1**, 152–3.

—— (1882).—" Ueber Licht- und Farbenperception niederster Organismen ", Pflüger's 'Arch. f. d. ges. Physiologie', **29**, 387–400.

Guillermond, A. (1921).—" Sur les éléments du cytoplasme chez les végétaux ; Chondriome, appareil vacuolaire et granulations lipoïdes ", 'Arch. de Biol.', **31**, 1–82.

Hardy, W. B. (1899).—" Structure of cell protoplasm ", 'Journ. Physiol.', **24**, 158–210.

Harrison, R. G. (1910).—" The outgrowth of the nerve fibre as a mode of protoplasmic movement ", 'Journ. Exper. Zool.', **9**, 787–848.

—— (1913).—" The life of tissues outside the organism from the embryological standpoint ", 'Trans. Congr. Amer. Phys. and Surg.', **9**, 63–76.

Head, H., and Rivers, W. H. R. (1908).—" A human experiment in nerve division ", 'Brain', **31**, 323–450.

Henneguy, L. F. (1898).—" Sur les rapports des cils vibratiles avec les centrosomes ", ' Arch. d'Anat. microsc.', **1**, 481–97.

Herlaut, M. (1913).—" Études sur les bases cytologiques du méchanisme de la parthénogenèse expérimentale chez les Amphibiens ", ' Arch. de Biol.', **28**, 505–608.

Hill, A. V. (1910).—" A new mathematical treatment of changes of ionic concentration in muscle and nerve under the action of electric currents, with a theory as to their mode of excitation ", ' Journ. Physiol.', **40**, 190–224.

Hoven, H. (1910).—" Sur l'histogenèse du système nerveux périphérique chez le Poulet et sur le rôle des chrondriosomes dans la neurofibrillation ", ' Arch. d. Biol.', **25**, 427–92.

Khainsky, A. (1910).—" Untersuchungen über Arcella ", ' Arch. f. Protistenkunde ', **21**, 164–83.

Knauthe, K. (1899).—" Beobachtungen über den Gasgehalt der Gewässer im Winter ", ' Biol. Centr.', **19**, 783.

—— (1898).—" Der Kreislauf der Gase in unseren Gewässern ", ibid., **18**, 785.

Lenhosek, M. v. (1898).—" Untersuchungen über Spermatogenese ", ' Arch. f. mikr. Anat.', **51**, 215–318.

Lucas, K. (1910).—" An analysis of changes and differences in the excitatory process of muscles and nerves based on the physical theory of excitation ", ' Journ. Physiol.', **40**, 225–49.

Macallum, A. B. (1905).—" On the distribution of potassium in animal and vegetable cells ", ibid., **32**, 95.

—— (1911).—" Oberflächenspannung und Lebenserscheinungen ", ' Ergebnisse d. Physiol.', **11**, 598–657.

Macdonald, J. C. (1902).—" The injury current of nerve the key to its physical structure ", ' Thomson Yates Lab. Reports ', **4**⁰ ii, 213–347. Liverpool Univ. Press.

—— (1905).—" The structure and function of nerve-fibres ", ' Proc. Roy. Soc.', **76 B**, 322–50.

Maupas, E. (1885).—" Sur Coleps hirtus (Ehrenberg) ", ' Arch. Zool. Exp. et Génér.', 2e série, **3**, 337.

Meves, F. (1900).—" Ueber den von la Valette St. George entdeckten Nebenkern (Mitochondrienkörper) der Samenzellen ", ' Arch. f. mikr. Anat.', **56**, 553–605.

—— (1903).—" Ueber oligopyrene und apyrene Spermien und über ihre Entstehung nach Beobachtungen an Paludina und Pygaera ', ibid., **61**.

—— (1908).—" Die Chondriosomen als Träger-Anlagen ", ibid., **72**.

Montgomery, T. H. (1911).—" The Spermatogenesis of an Hemipteron Euschistus ", ' Journ. of Morph.', **22**, 731–98.

Nernst, W. (1899).—" Zur Theorie der electrischen Reizung ", ' Götting. Nachrichten, Math.-phys. Kl.', 104–8.

Ostwald, Wolfgang (1908).—" Ueber die Lichtempfindlichkeit tierischer Oxydasen und über die Beziehungen dieser Eigenschaft zu den Erscheinungen des tierischen Phototropismus ", ' Bioch. Zeitschr.', **10**, 1–130.

Petry, M. (1849).—" Eine physiologische Eigenthümlichkeit der Rhizopodensippe Arcella Ehr.", ' Mitt. d. Naturf. Ges. in Bern', 124–6.

—— (1852).—' Zur Kenntniss kleinster Lebensformen nach Bau, Funktion, Systematik, mit Specialverzeichniss der in der Schweiz beobachteten ', Bern.

Robertson, M., and Minchin, E. A. (1910).—" The division of the collar-cells of Clathrina coriacea (Montagu) : A contribution to the theory of the centrosome and blepharoplast", 'Quart. Journ. Micr. Sci.', **55**, 611–40.

Shearer, C. (1922).—" On the oxidation of the Echinoderm egg during fertilization ", ' Proc. Roy. Soc.', B **93**, 213–29.

Vernon, H. M. (1911).—" The quantitative estimation of the indophenol oxidase of animal tissues ", ' Journ. of Physiol.', **42**, 402.

Verworm, M. (1909).—' Allgemeine Physiologie ', 5te Auflage.

Wager, H. (1900).—" On the eye-spot and flagellum of Euglena viridis ", ' Journ. Linn. Soc. London, Zool.', **27**, 23.

Warburg, O. (1913).—' Ueber die Wirkung der Struktur auf chemische Vorgänge in Zellen.' Jena.

Wilson, C. B. (1898).—" Activities of mesenchyme in certain larvae ", ' Zool. Bull.', **2**, 15–23.

Wolff, J. (1910).—' Contributions à la connaissance de divers phénomènes oxydasiques naturels et artificiels.' Laval, Barnéoud.

Zuntz, N. (1900).—" Ueber den Kreislauf der Gase in Wasser ", ' Arch. f. Anat. und Physiol.', Suppl., 311.

EXPLANATION OF PLATES.

PLATE 30.[1]

Fig. 1.—Magnification 300 diameters. A. Side view of a reversed Arcella. A–E shows the movements of its pseudopodia, and the use of one of them to right itself. F, the Arcella righted and crawling on the surface of the glass. (For full description see p. 541.)

Fig. 2.—Magnification 800 diameters. Diagrammatic sketches of a portion of the marginal zone of Arcella, showing the movements of the pseudopodia before, during, and after the formation of a minute gas-vacuole. No distinction has been made in the diagram between ectoplasm and endoplasm, and the whole of the protoplasm has been uniformly stippled. The times, from 4 min. 15 sec. to 14 mins. are reckoned from the time of reversal with a needle as zero time. *m.p.*, marginal pseudopodium ;

[1] All the figures of Plates 30–5 are drawn from Arcella discoides Ehrenberg.

m.s., marginal space; *g.v.*, gas-vacuole; *c.v.*, contractile-vacuole. (For full description see p. 543.)

PLATE 31.

Fig. 3.—Magnification 850 diameters. A young daughter Arcella drawn from life after reversal. Note the small bulk of protoplasm and large marginal space. Three external pseudopodia: $e.p.^7$, $e.p.^{11}$, $e.p.^3$. Karyosome (K) in the nuclei. The single gas-vacuole ($g.v.^5$) at the base of a large hyaline lobopod bearing filopodia. *n.*, nucleus; *f.v.*, food-vacuole; $m.p.^7$ and $m.p.^{10}$, marginal pseudopodia; $c.v.^9$, contractile-vacuole. (For full description see p. 540.)

Fig. 4.—Magnification 900 diameters. A diagrammatic optical section of Arcella. The only parts of this diagram drawn from the object are the shape of the test and the shape of the pseudopodia, which were drawn from a reversed Arcella about two minutes after reversal. The details of the protoplasm in the test are reconstructed from a large number of observations taken from the observe and reverse sides. (For full description see p. 538.)

PLATE 32.

Fig. 5.—A–P. Magnification 800 diameters. A series of sixteen diagrams of the marginal zone of a daughter Arcella reversed under the conditions of the standard experiment. The condition of the marginal protoplasm before, during, and after the appearance of two gas-vacuoles is shown. The protoplasm is uniformly stippled without discriminating between endoplasm and ectoplasm. The times indicated are taken from the time of reversal as zero time. (For full description see p. 545.)

PLATE 33.

Fig. 6.—Magnification 700 diameters. An Arcella killed in cold saturated corrosive sublimate stained with borax carmine and mounted in Canada balsam. The specimen was killed with contractile vacuoles ($c.v.^1$, $c.v.^5$, $c.v.^7$, and $c.v.^9$) still expanding, and fixed, as in life, without contraction. The pseudopodia are preserved almost without contraction. A narrow deeply stained zone in the marginal region shows the position of what has been described as chromidia.

Fig. 7.—Magnification 700 diameters. An Arcella killed in 4 per cent. formaldehyde solution, passed through 30 per cent. and 50 per cent. alcohol, and stained in Ehrlichs haematoxylin and mounted in a mixture of equal parts of glycerin water and 90 per cent. alcohol. The gas in the vacuoles ($g.v.^{12}$, $g.v.^2$, $g.v.^5$, $g.v.^9$) after this treatment remained undiminished in volume. The drawing was made more than seven minutes later, when the gas in the four vacuoles had only slightly diffused out. There is a large external pseudopodium ($e.p.^4$) spread over the test from iii to v. (For full description see p. 582.)

PLATE 34.

Fig. 8.—Magnification 800 diameters. Arcella stained for 5 min. in a weak solution of methylene blue and then reversed. It shows growing gas-vacuoles; the cytoplasm is distinctly blue in the outer zone, but pale in the central mass. The two small gas-vacuoles are represented in the act of expanding. The karyosome and the radiating filaments have turned pale blue. (See p. 564.)

Fig. 9.—Magnification 800 diameters. Arcella containing two large and one small vacuole at ix, vii, and v killed and stained with saturated solution of benzidine in a weak alcohol. The depth of stain varies in different parts of the cytoplasm, but the colour is much deeper in the marginal zone of protoplasm. The central mass is completely devoid of benzidine granules. (See p. 584.)

PLATE 35.

Fig. 10. A–N. Magnification 800 diameters. Sketches of the marginazone of an Arcella treated with methylene blue, showing the last stages of a gas-vacuole before its disappearance. The drawings were made from the same vacuole, and show alternate expansion and contraction accompanied by deeper staining of the protoplasm during contraction and bleaching of the protoplasm during expansion. A, gas-vacuole beginning to contract; B, vacuole fully contracted; C, expanding vacuole; D, gas-vacuole almost fully expanded; E, contracting vacuole indicating reticular structure in protoplasm very clearly; F, expanding gas-vacuole; G, gas-vacuole beginning to contract; H, contracted gas-vacuole; I, expanded gas-vacuole; J, contracted gas-vacuole; K, expanded gas-vacuole; L, final contraction before disappearance; M, clear patch of protoplasm in the space occupied by the vanishing gas-vacuole; N, the bleached and contracted protoplasm a few minutes after the disappearance of the gas-vacuole. (For full description see p. 564.)

PLATE 36.

Fig. 11.—Paramecium caudatum Ehrbg. killed with saturated corrosive sublimate; washed in 30 and 50 per cent. alcohol and stained with a saturated solution of benzidine in 50 per cent. alcohol (made with absolute alcohol) to which 5 per cent. glycerol had been added. The blue dots covering the whole surface are the basal bodies of the cilia. Note the large greenish macronucleus, and to the left of it the single micronucleus. (For full description see p. 610.)

Fig. 12.—Magnification 2,250 diameters (compensating eye-piece 18, with 2 mm. Apo. objective). The macronucleus of the specimen drawn in fig. 11 showing the benzidine oxidase in the micronucleus and the scattered benzidine oxidase granules in the deeper endoplasm. A small piece of the

macronucleus is shown on the left; it contains no benzidine oxidase, but has reacted to form a green colour product with the benzidine. (For full description see p. 611.)

Fig. 13.—Paramecium caudatum Ehrbg. Outline sketch to show the position of the portions of the surface shown under high magnification in figs. 13 A, 13 B, and 13 C.

Fig. 13 A.—Magnification 2,250 diameters. Paramecium caudatum. A part of the surface of the endoplasm near the posterior end, showing the basal bodies stained deep blue with benzidine and connected by bands of less deeply stained oxidase. The patch is orientated on the plate as it is in fig. 13, so that the longitudinal connecting strands running from right to left are antero-posterior. (For full description see p. 611.)

Fig. 13 B.—Magnification 2,250 diameters. Paramecium caudatum. A part of the surface of the endoplasm from the edge of the peristome. The upper part lying inside the groove, the lower and more regular arrangement of basal bodies is on the edge of the groove. The isolated dots lying singly or in pairs in the meshes of the connecting strand are strands of the deeper lying oxidase reticulum of the endoplasm seen end on. Here and there can be seen a faint indication that the internal reticulum is connected with the basal bodies by fine strands of benzidine oxidase. This figure again is orientated on the plate as in fig. 13. (For full description see p. 611.)

Fig. 13 C.—Magnification 2,250 diameters. Paramecium caudatum. The part of the surface near the anterior end, showing the basal bodies stained with benzidine blue with the connecting strands of benzidine oxidase. Each cilium can be traced to its own basal body. (For full description see p. 611.)

PLATE 37.

Fig. 14.—Magnification 3,600 diameters. Coleps hirtus Ehrbg. Killed with saturated corrosive sublimate, washed in 30 and 50 per cent. alcohol, and stained with a saturated solution of benzidine in 50 per cent. alcohol, to which 5 per cent. glycerol had been added. The basal bodies and a portion of the superficial network of oxidase substrate granules, and connecting strands of the same nature have been drawn. The internal network of oxidase strands is not shown. In the region of the nucleus, the connexion between basal bodies through the superficial network has been drawn. In the macronucleus and the large kidney-shaped micronucleus lying behind it, there is a network of oxidase granules with fine connecting strands, all stained with benzidine blue much more precisely in the micronucleus than in the macronucleus. (For full description see p. 612.)

PLATE 38.[1]

Fig. 15.—Lumbricus herculeus Rosa. A cell from the edge of the nephridial funnel of an anterior segment. The cell is shown in optical section. The nephridium was dissected out in normal salt solution, killed with saturated corrosive sublimate, washed in 30 and 50 per cent. alcohol, and stained with a saturated solution of benzidine in 50 per cent. alcohol (made with absolute alcohol) to which 5 per cent. glycerol was added. The cell is drawn in optical section in a plane containing the nucleus and nucleolus. The basal bodies are connected by fine strands of oxidase with the oxidase network which fills the whole of the cell throughout the cytoplasm, the nucleus, and the nucleolus. The network of the reticula in the nucleus are larger than those in the cytoplasm, and uniform in size with those in the nucleolus. The nucleolus appears to contain a large mass of oxidase substrate, less deeply stained than the bodies at the nodes of the network. The base of each cilium appeared to be stained with benzidine blue, but it was impossible to decide whether this was not due to the effect of reflection and refraction of the light which had passed through the stained deeper portion of the nephridium. (For full description see p. 614.)

Fig. 16.—Primary spermatocyte from a smear preparation of the testis of a rat. The preparation was made within ten minutes of the death of the rat, the tissues being kept at the body temperature during killing and dissection. The smear was hardened in saturated corrosive sublimate, washed in 30 and 50 per cent. alcohol, and stained in a saturated solution of benzidine in 50 per cent. alcohol (made with absolute alcohol), to which 5 per cent. glycerol had been added. The whole of the cell is filled (a portion only has been drawn) with a coarse network of oxidase substrate, more deeply stained at the nodes than in the strands connecting the nodes. Two astrospheres have formed, and have taken a deep benzidine blue stain. The lower of the two shows two long fibres passing to the equator of the nucleus, and these may be two of the sixteen spindle-threads. (For full description see p. 621.)

Fig. 17.—Spermatid from the same preparation as the above, stained with picrocarmine after treatment with benzidine. The cytoplasm formed a blunt cone, the first stage in the development of the tail. Two centrosomes deeply stained with benzidine blue lie close to the nucleus, and another pair of similar bodies similarly stained are seen inside the nucleus at opposite sides of the equator. A very coarse network of oxidase is contained in the nucleus, which also contains an acrosome body. Both the acrosome and the nucleus are filled with diffused chromosome substance

stained by the picrocarmine. The cytoplasm is filled with a delicate network of oxidase condensed towards the apex of the tail-cone. (For full description see p. 620.)

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[1] All the preparations shown on this plate were stained for about 2 hours in the benzidine solution, and were then gradually passed into a mixture of equal parts of glycerol water and 90 per cent. alcohol, by running this under the cover glass. As the alcohol evaporated, the loss was made up by adding pure glycerol.

Fig. 18.—A primary spermatocyte from the same preparation as the above at the end of the prophase of mitosis. The astrospheres and the spindle-threads, each ending in a divided chromosome, and the chromosomes themselves, are all stained with benzidine blue. The chromosomes and spindle-fibres of the upper side of the spermatocyte (eight in number) have been drawn, the other eight of the total sixteen were counted on the other surface of the spherical cell. Besides the spindle-figure the cell contained irregular masses and large granules of oxidase, all of which were in focus near the upper surface. (For full description see p. 616.)

Plate 39.

Fig. 19.—Developing spermatozoan of rat from the same preparation as the above (figs. 16–18) stained with benzidine only. The two centrosomes have moved into the cone of cytoplasm now developing into the tail. Of these two centrosomes the proximal one is secreting the axial fibre which passes through the ring-shaped distal centrosome. Posterior to the centrosome is a mass of oxidase which distally is developing into the spiral band of the middle piece. The cytoplasm is filled with a network of oxidase with the nodes stained exactly like the centrosomes. The nucleus contains a similar network. (For full description see p. 617.)

Fig. 20.—The same specimen as that shown in fig. 19 after staining with picrocarmine drawn at a different focus. The chromatin is stained pink and is diffused through the cytoplasm with the exception of the developing middle-piece. The chromatin seems to have entirely diffused out from the nucleus. At the centre of the nucleus is a large mass of oxidase with a roughly radial structure. (For full description see p. 618.)

Fig. 21.—Developing spermatozoan of mouse. A later stage in the development of the spermatozoan than that shown in fig. 20, drawn from a smear preparation of mouse testis exactly as the rat testis smear described above (fig. 16), stained with benzidine and picrocarmine. The chromatin is diffused through the cytoplasm of the head. The portion of the cytoplasm which is cast off is being constricted from the tip of the head. The cytoplasm of the middle-piece has extended down the axial fibre, and the oxidase is forming the spiral band in it. (For full description see p. 618.)

Fig. 22.—Head and part of the middle-piece of a fully developed spermatozoan of rat drawn from the same preparation as figs. 16, &c., stained with benzidine only. The head contains diffused oxidase; the two chromosomes, the connecting strand of oxidase, and the spiral band, have taken the characteristic benzidine blue stain. (For full description see pp. 615–16.)

Plate 40.

Fig. 23.—‘ Seven successive views of the end of growing nerve-fibre, showing its change of shape and progression. Red blood corpuscles shown in outline marks the fixed point ’—after Ross G. Harrison (1913, fig. 8). The time intervals of the original are here adapted to zero time.

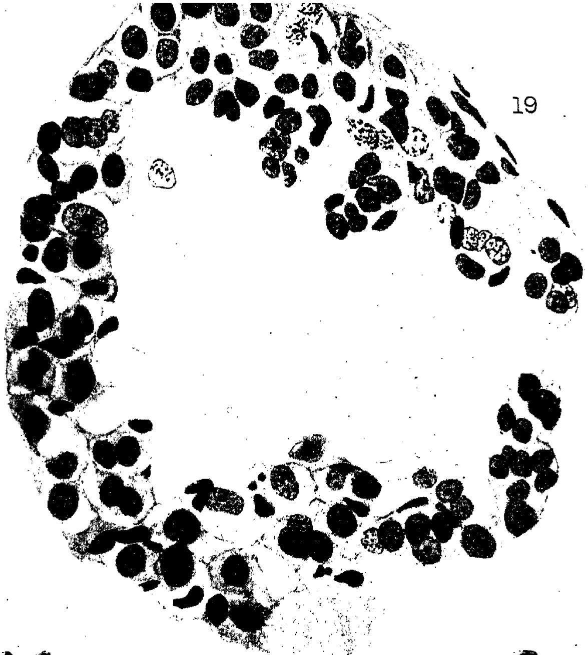
Fig. 24.—‘ A small part of a blastula of the starfish common at Roscoff, France, reduced one-half from a camera sketch made by G. F. Andrews in 1894 with oc. 8, obj. 2 mm., tube 170 mm.’—after E. A. Andrews (1898, Text-fig. 1).

Fig. 25.—First maturation spindle in a spermatocyte floating free in the testicular cavity of Euschistus variolarius Pal. Beauv. After T. H. Montgomery, Jun. (1911, Pl. 3, fig. 89). The chondriosome filaments shown in the original drawing are here omitted.

Fig. 26.—Spermatocyte of a normal spermatozoan of Pygaera bucephala with first maturation spindle. The centrosomes lie immediately under the cell surface (p. 567). The spermatocyte is fixed to the wall of the testis, and the flagella protrude into its lumen. After F. Meves (1900, Pl. 27, fig. 55).

Fig. 27.—Magnification 4,000 diameters. Collar-cell of Clathrina coriacea (Montagu) ‘ with daughter flagella growing out from the centrosomes (blepharoplasts) ’. After Muriel Robertson and E. A. Minchin (1910, Pl. 25, fig. 50). Spindle-fibres not shown in the original drawing have been added in this figure.

Fig. 28.—Spermatocyte of Bombyx mori after E. F. Henneguy (1898, Text-fig. 1, p. 483).



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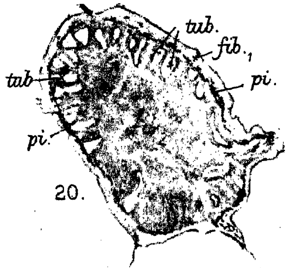

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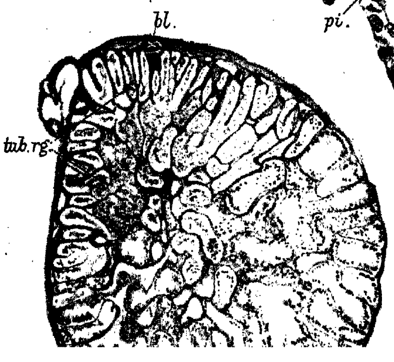

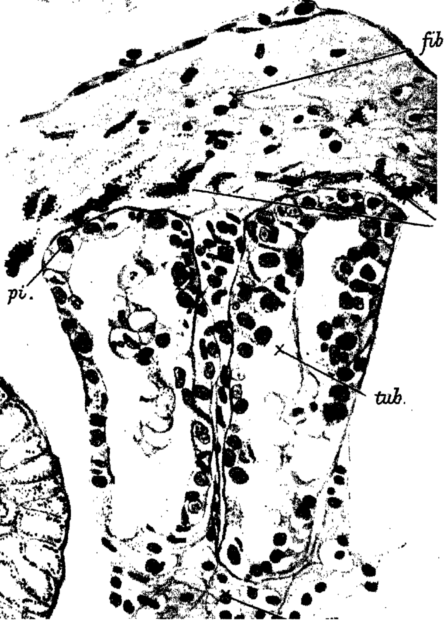

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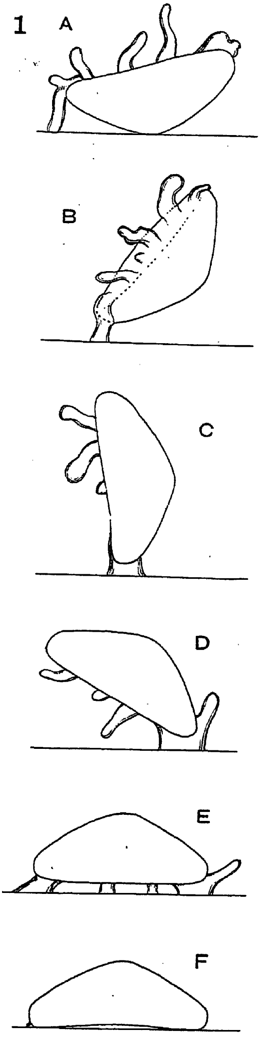
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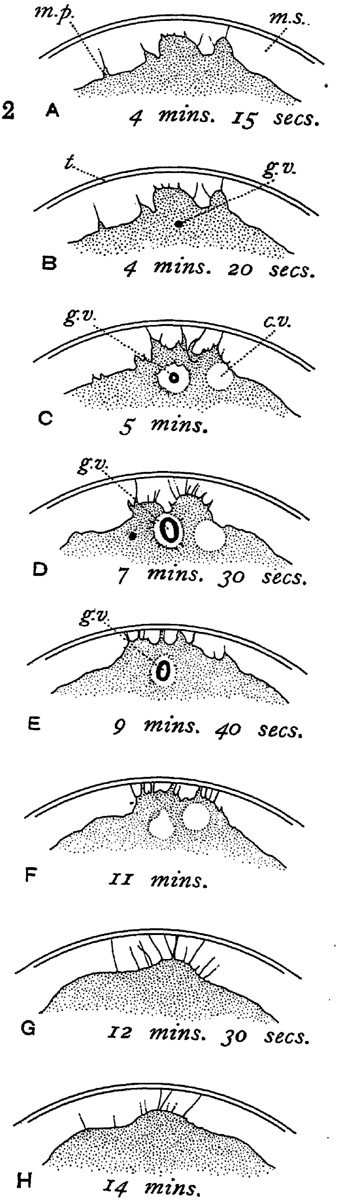
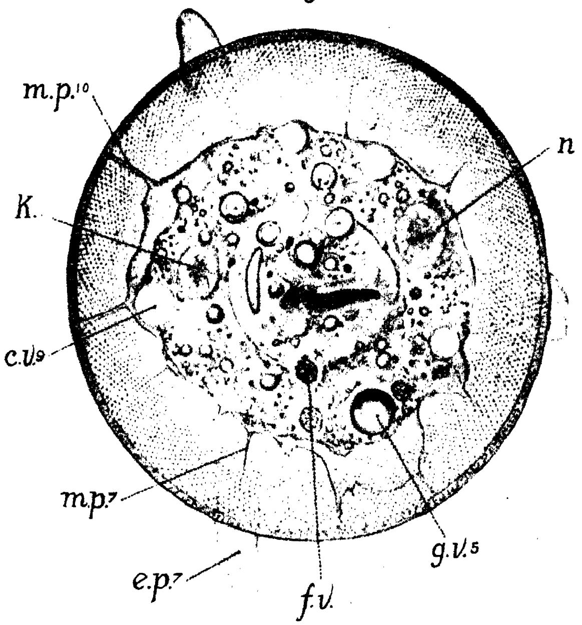
F

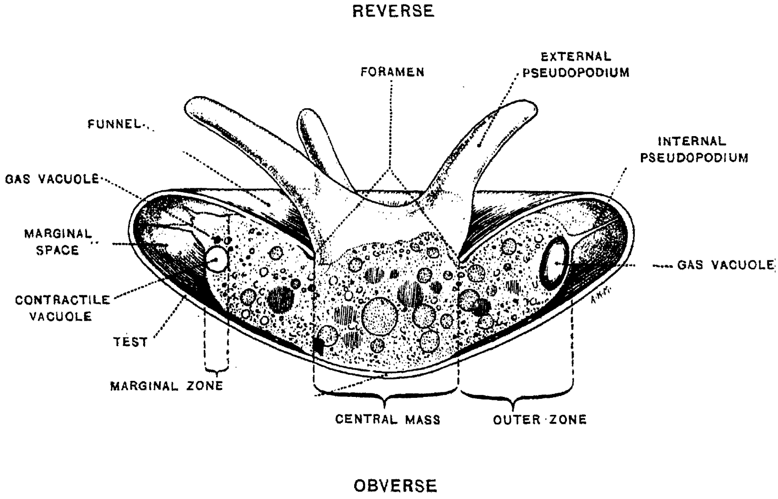
2


m.p.
m.s.
A
4 mins. 15 secs.
t.
g.v.
B
4 mins. 20 secs.
g.v.
c.v.
C
5 mins.
g.v.
D
7 mins. 30 secs.
g.v.
E
9 mins. 40 secs.
F
11 mins.
G
12 mins. 30 secs.
H
14 mins.

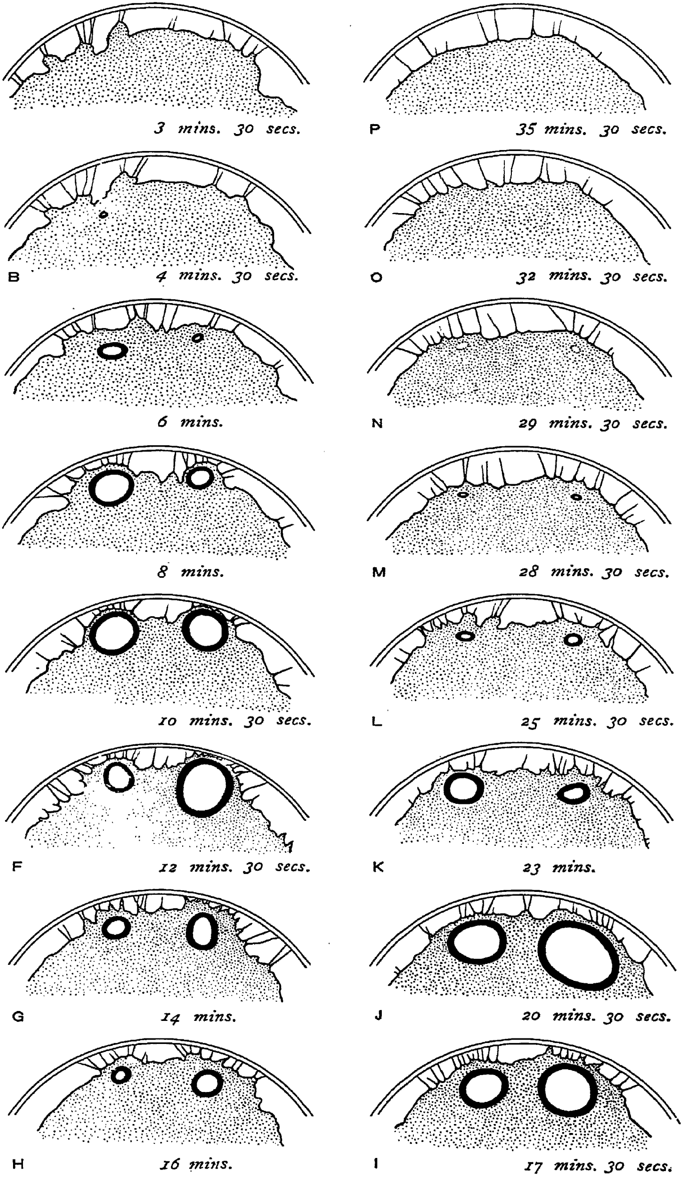
3


m.p.10
n
K.
c.v.9
m.p.7
e.p.7
f.v.
g.v.5

4


REVERSE
FORAMEN
EXTERNAL PSEUDOPODIUM
FUNNEL
INTERNAL PSEUDOPODIUM
GAS VACUOLE
MARGINAL SPACE
GAS VACUOLE
CONTRACTILE VACUOLE
TEST
MARGINAL ZONE
CENTRAL MASS
OUTER ZONE
OBVERSE

5



3 mins. 30 secs.

P *35 mins. 30 secs.*

B *4 mins. 30 secs.*

O *32 mins. 30 secs.*

6 mins.

N *29 mins. 30 secs.*

8 mins.

M *28 mins. 30 secs.*

10 mins. 30 secs.

L *25 mins. 30 secs.*

F *12 mins. 30 secs.*

K *23 mins.*

G *14 mins.*

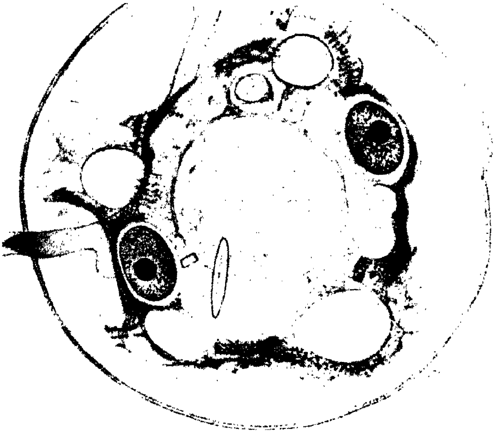
J *20 mins. 30 secs.*

H *16 mins.*

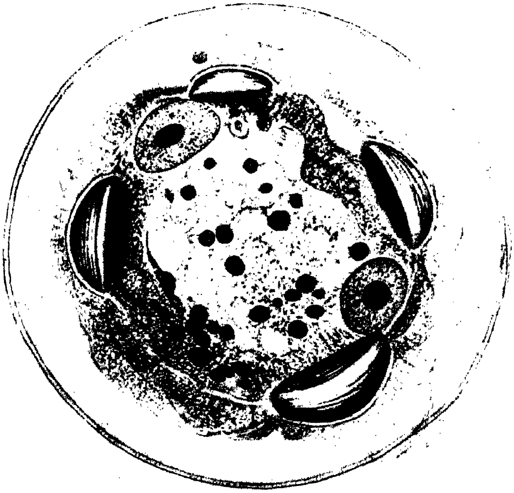
I *17 mins. 30 secs.*

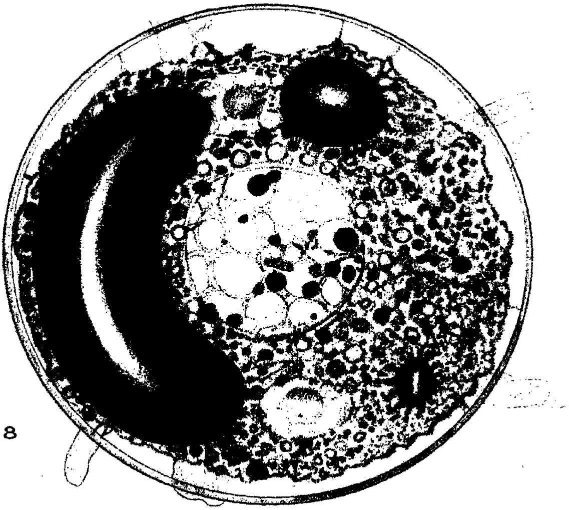
A. K. Maxwell, del.

6

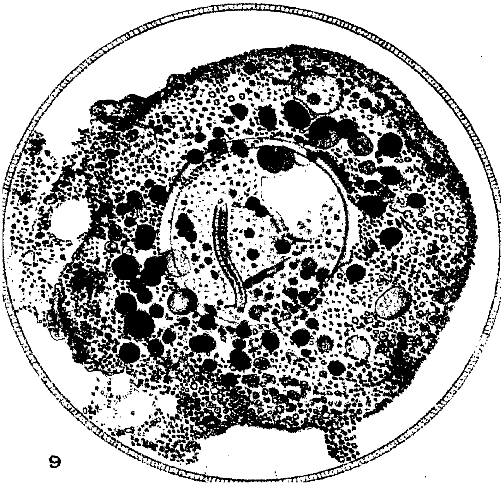


7





8



9

A.K. Maxwell del.

Huth, London.